POSITIVE EFFECTS OF *ANDROGRAPHIS PANICULATA* EXTRACT ON GROWTH PERFORMANCE, HEMATOLOGY, SERUM BIOCHEMISTRY, ORGANOSOMATIC INDICES AND PATHOGENIC CHALLENGE TEST WITH *AEROMONAS HYDROPHILA* IN HYBRID CATFISH (*CLARIAS MACROCEPHALUS X C. GARIEPINUS*)

EAR CHITRA

A Thesis Submitted to University of Phayao

in Partial Fulfillment of the Requirements

for the Master of Science Degree in Fisheries Technology and Innovation

April 2024

ผลในเชิงบวกของสารสกัดฟ้าทะลายโจรต่อประสิทธิภาพการเจริญเติบโตค่าโลหิตวิทยา ค่าชีวเคมี ดัชนีอวัยวะ และการทดสอบความท้าทายของเชื้อโรค *Aeromonas hydrophila* ในปลาดุกลูกผสม (*Clarias macrocephalus x Clarias gariepinus*)

Ear Chitra

วิทยานิพนธ์เสนอมหาวิทยาลัยพะเยา เพื่อเป็นส่วนหนึ่งของการศึกษา
หลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีและนวัตกรรมการประมง
เมษายน 2567

POSITIVE EFFECTS OF *ANDROGRAPHIS PANICULATA* EXTRACT ON GROWTH PERFORMANCE, HEMATOLOGY, SERUM BIOCHEMISTRY, ORGANOSOMATIC INDICES AND PATHOGENIC CHALLENGE TEST WITH *AEROMONAS HYDROPHILA* IN HYBRID CATFISH (*CLARIAS MACROCEPHALUS X C. GARIEPINUS*)

EAR CHITRA

A Thesis Submitted to University of Phayao

in Partial Fulfillment of the Requirements

for the Master of Science Degree in Fisheries Technology and Innovation

April 2024

Thesis

Title

POSITIVE EFFECTS OF *ANDROGRAPHIS PANICULATA* EXTRACT ON GROWTH PERFORMANCE, HEMATOLOGY, SERUM BIOCHEMISTRY, ORGANOSOMATIC INDICES AND PATHOGENIC CHALLENGE TEST WITH *AEROMONAS HYDROPHILA* IN HYBRID CATFISH (*CLARIAS MACROCEPHALUS X C. GARIEPINUS*)

Submitted by EAR CHITRA

Approved in partial fulfillment of the requriements for the
Master of Science Degree in Fisheries Technology and Innovation
University of Phayao

Approved by

---------- Chairman
(Associate Professor Dr. chanagun Chitmanat)

---------- Advisor
(Associate Professor Dr. Paiboon Panase)

---------- Co Advisor
(Dr. Korntip Kannika)

---------- Examiner
(Dr. Sontaya Sookying)

---------- Examiner
(Associate Professor Dr. Chatmongkon Suwannapoom)

---------- Dean of School of Agriculture and Natural Resources
(Assistant Professor Dr. Wipornpan Nuangmek)

Title: POSITIVE EFFECTS OF *ANDROGRAPHIS PANICULATA* EXTRACT ON GROWTH PERFORMANCE, HEMATOLOGY, SERUM BIOCHEMISTRY, ORGANOSOMATIC INDICES AND PATHOGENIC CHALLENGE TEST WITH *AEROMONAS HYDROPHILA* IN HYBRID CATFISH (*CLARIAS MACROCEPHALUS X C. GARIEPINUS*)

Author: Ear Chitra, Thesis: M.Sc. (Fisheries Technology and Innovation), University of Phayao, 2023

Advisor: Associate Professor Dr. Paiboon Panase Co-advisor Dr.Korntip Kannika

Keywords: Andrographis paniculata Growth performance Organosomatic indices Hematological indices Biochemical indices Hybrid catfish

ABSTRACT

This research was conducted to determine the effects of *Andrographis paniculata* extract on growth performance, hematological, organosomatic and biochemical indices of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*). The fish were fed 0, 0.2, 0.4 and 0.6 g/kg for 90 days. The results showed that the with the highest weight gain (WG), average daily growth (ADG), specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER), as well as the lowest feed conversion ratio (FCR), were found in the group fed with *A. paniculata* extract at 0.6 g/kg. However, survival rate was not shown to be significantly different throughout the experiment. The hematological indices, total red blood cells (RBC) was at its highest in all treatment groups, while the highest levels of total white blood cells (WBC) and hematocrit (Htc) were observed in the group fed 0.6 g/kg when compared with other groups. Serum alanine transaminase and triglyceride were at their lowest levels in all treatment groups, while the highest levels of serum glucose were found in all treatment groups. The highest levels of high-density lipoprotein and the lowest level of cholesterol were observed in fish fed 0.6 g/kg, while other biochemical parameters were not significantly different ($P > 0.05$). Lysozyme activity was initially observed at a significant difference on days 60 – 90, where the highest activity was found in the group fed 0.6 g/kg. All of organosomatic indices showed no significant differences. After 14 days of *Aeromonas hydrophila* challenge test, serum lysozyme activity and WBC were significantly increased in all treatment groups, while the lowest cumulative mortality was exhibited in the group fed 0.6 g/kg, when compared to other groups. These results suggest that 0.6 g/kg of *A. paniculata* can improve growth performance, hematological indices, biochemical indices, disease resistance and non-specific immune responses of hybrid catfish.

ACKNOWLEDGEMENT

I am particularly grateful to the Royal Scholarship 2564 under Her Royal Highness Princess Maha Chakri Siridhon Education Project to the Kingdom of Cambodia.

I am especially thankful to my advisor, Assoc. Prof. Dr. Paiboon Panase for his constant Support, insightful comments, constructive critisms, inspiring guidance and encouragement throughout this work. I would also like to thank Asst. Prof. Dr. Sontaya Sookying, Dr. Korntip Kannika and Assoc. Prof. Dr. Chatmongkon Suwannapoom for their valuable advice, Suggestion and comments.

My study would not have been successful without the scholaship from the Thailand science research and innovation fund and the University of Phayao (Grant No. FF66 – UoE014).

We would like to thank the School of Agriculture and Natural Recourses and School of Pharmaceutical Sciences, at the University of Phayao, Thailand, for providing facilities for the present research. Moreover, the authors would like to thank all the staff and students in the fisheries program for their help with data collection.

Finally, I am very grateful to have a great family with enormous support, love and appreciation which gave me strength on my way.

Ear Chitra

LIST OF CONTENTS

Page

LIST OF TABLLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Background and Rationale of the Study

Hybrid catfish are produced from female Asian catfish, *Clarias microcephalus*, and male African catfish, *C. gariepinus*. The culturing of this fish is rapidly gaining in popularity in Southeast Asia due to its rapid growth, resistance to disease, The possibility for high stocking density, hypoxia tolerance and excellent meat quality. This hybrid is an important aquaculture and economically important worldwide (Chaivichoo et al., 2020). Historically, hybrid catfish have been in high demand in Southeast Asian countries such as Thailand, the Philippines, Indonesia, Malaysia and Cambodia. In Thailand, the production of this hybrid catfish was approximated to be over 100,000 tonnes, with over 150 million USD in 2018 (FAO, 2020). However, when there is a demand for high productivity, fish aquarists often use a high-density farming system, which can make fish stressed, grow slowly, become easily infected with disease, eventually die which in turn causes huge economic losses (Quesada et al., 2013; Bulfon et al., 2015). In preliminary problem-solving, farmers use chemicals and antibiotics to prevent and treat infections, which, in the early stages of treatment, can be quite effective (Harikrishnan et al., 2011; Kamaraj et al., 2018). Prolonged use of antibiotics can lead to the progression of antibiotic-resistant pathogens. Also, chemicals left as residues in the fish are dangerous for human consumption and cause harmful bioaccumulation impacts (Hidayat et al., 2018). Nowadays, several plants or their by-products are being used instead of antibiotics; these are regarded as being safer since they contain many organic bioactive components such as phenolic, polyphenolic, alkaloid, quinone, and essential oils (Chakraborty et al., 2014; Tan et al., 2018). Panase et al. (2018a). For example, it has been shown that the extract of *Euphorbia hirta* can have an effect on growth parameters, hematological indices and organosomatic indices on hybrid catfish *Clarias macrocephalus x C. gariepinus*. Moreover, with regards to hybrid catfish, *C. macrocephalus x C. gariepinus, Houttuynia cordata* extract was shown to promote growth and hematological

indices (Panase et al., 2018b). In addition, *Apium graveolens* plant extract sprayed on feed, was shown to improve growth performance, serum biochemical indices and lysozyme activity indices of *Labeo chrysophekadion* (Sutthi et al., 2020). The medicinal herb *Andrographis paniculata*, which is tropical and sub-tropical Asia, Southeast Asia, and India and has a greatly bitter taste, is used to cure liver disorders, children's bowel complaints, colic discomfort, the common cold and upper respiratory infections (Jayakumar et al., 2013; Hossain et al., 2014). The aerial part of *A. paniculata* is commonly used in Chinese medicine where it is believed to cool and relieve internal heat, inflammation, pain and against cancer as a form of detoxification (Gupta et al. 2017; Suriyo et al. 2021; Valdiani et al. 2022). The major bioactive compounds of *A. paniculata* are andrographolide deoxy andrographolide, neo andrographolide, 14-deoxy-11,12-didehydroandrographide and is andrographolide respectively (Valdiani et al. 2017). When their feed is supplemented with (*A. paniculata*) aqueous methanolic extract at a dose of 2%, *Pangasianodon hypopthalmus* can show improved growth indices, immunity and prevention of *Aeromonas hydrophila* infection, lysozyme activity, total white blood cell, red blood cell, hemoglobin content and hematocrit (Maiti et al., 2021). However, the effect of *A. paniculata* on hybrid catfish has not yet been reported.

Therefore, the objective of this study was to investigate *A. paniculata* extract's effectiveness in supplementing growth performance, biochemical indices, hematological indices, organosomatic indices, innate immunity and bacterial disease resistance against *A. hydrophila* in hybrid catfish, *C. macrocephalus* × *C. garipinus*.

CHAPTER 2

LITERATURE REVIEW

2.1 Kariyat, *Andrographis paniculata* (Burm.f.) Nees.

Figures 1 Andrographis paniculata

2.1.1 Botanical description of the *Andrographis paniculata*

Common name: Kariyat, The Creat

Family name: Acanthaceae

Scientific name: *Andrographis paniculata*

Local name: Fa thalai, Fa thalai chon, Ya kan ngu (Thailand); Si–Pang–ki (Chinese)

A. paniculata is an economically important herb of the genus *Andrographis*. It is an erect and branched and annual flowering herb. *A. paniculata* is an important medicinal plant and widely used around the world. It is a member of the *Acanthaceae* family. *A. paniculata* is a traditional herbal medication used in Bangladesh, China, Hong Kong, India, Pakistan, Philippines, Malaysia, Indonesia and Thailand to cure snake bite, insect bite, diabetes, diarrhea, fever, and malaria. The genus *Andrographis* has 40 species, 19 of

which are known to be available in India, with the therapeutic qualities of *A.paniculata* and *Andrographis alata*. This plant, when grown, may reach a height of 30 to 110 cm. Its stem is dark green, 30–110 cm long, 2 to 6 mm in diameter, quadrangular with longitudinal furrows and wings at angles of the juvenile sections, and somewhat increased at the nodes. The leaves are dark green, glabrous, 2–12 cm long and 1–3 cm wide, opposite, decussate, lanceolate, with an entire border and pinnate venation; the petiole is extremely short (Figure 2.1). The blooms are tiny, with five linear particle calyces, a short tube, and a 6 mm long white corolla with rose–purple dots on the petals. The blooming and fruiting season runs from December to April. The fruits are little 2–celled odorless erected capsules, 1–2 cm long, 2–5 mm broad, linear–oblong, and acute at both ends. Different extracts and their secondary metabolites, particularly andrographolide, are one of the extensively studied natural products. The therapeutically active extracts are prepared, or metabolites isolated from aerial parts, leaves, roots, whole plants or calluses (Shalini and Narayanan 2015).

2.1.2 Bioactive compounds of *A. paniculata*

Andrographolide, neoandrographolide and isoandrographolide are the most abundant lead bioactive compounds that can be isolated from any part of *A. paniculata* For example, aerial apart, leaves, whole plant, and even roots. However, these compound are present in high amounts in leaves. The yield reached the maximum level while the plant materials are collected between 110–130 days of cultivation (Table 2.2) (Sharma et al., 2013). In addition, (Cheung et al., 2001) reported that the percentage content of andrographolide, deoxyandrographolide, and neoandrographolide was found in the whole plant, leaf, stem, and root of *A. paniculata* (Table 2.3). Pandey and Mandal (2010) reported that the average andrographolide content varied from 1.07 to 2.24 percent in dried leaves. Furthermore, *A. Paniculata* contains therapeutically active secondary metabolites that include lactones, diterpenes, flavonoids, quinic acid, xanthones, noriridoids, and other compounds (Hossain et al., 2014). In addition, Nagajothi et al., (2018) reported that the *A. Paniculata* extract wiht aqueous and ethanolic contains bioactive compound such as alkaloids, cardiac glycosides, phenols, tannins, phlobatannins, hydrolysable tannins,flavonoids, terpenoids and saponins (Table 2.1). However, Rajalakshmi and Cathrine (2016) reported that the the analysis of phytochemicals in

the ethanolic extracts of *A. paniculata* was presence bioactive compound such as alkaloids on alkaloids (Mayer's reagent), carbohydrates and glycosides (Molisch's test), sugar (Benedict's reagent), saponin (Foam test), protein (Millon's test), phytosterol (Libermann Burchard's test) phenolic compounds and tannin (Ferric chloride test), flavonoid (Alkaline reagent test) and glycosides (Legal's test) (Table 2.4).

2.1.3 Medicinal properties of *A. paniculata*

Properties of *A. paniculata* is reported to be an antioxidant and immuno stimulant. The aqueous extract of *A. paniculata* showed significant antibacterial activity, due to the presence of andrographolides and arabinogalactan proteins. Andrographolide and neoandrographolide play role in treating bacillary dysentery caused by *Shigella sp.* (Sivananthan and Elamaran 2013). Moreover, andrographolide and neoandrographolide can resist bacteria, viruses, eliminate parasites and treat diarrhea caused by *E. coli* infection (Gupta et al., 1990). Furthermore, andrographolide can be used for respiratory treatments such as colds and fever (Caceres et al., 1997). Besides, dehydroandrographolide and andrographlide can cure inflammation, treat gastrointestinal diseases, stimulate bile secretion, increase digestion, relax muscle, lowe blood sugar, inhibit platelet aggregation, prevent blood clots, prevent liver damage and stimulate the immune system (Ellis et al., 1978). In addition, the amount of active ingredient will vary depending on the extraction method. *A. paniculata* is effective in anti–inflammatory. kill bacteria Stimulates immunity by increasing the efficiency of white blood cells to destroy foreign matter prevents blood clotting, inhibits viruses, inhibits and destroys cancer cells (Table 2.5). make digestion better reduces fever and has the effect of contracting muscles (Cheung et al., 2001; Bhaskar–Reddy et al., 2003). In addition, Sahalan et al. (2007) m ethanol extract of leaves showed significant activity against *E. coli* along with *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis* and *S. epidermidis*. However, ethanol extracts were found effective against *Legionella pneumophila* and *Bordetella pertussis* only. Thus, the extraction process and solvent have a significant role in the efficacy of *A. paniculata* as the number and yield of pure metabolites greatly differ depending on the types of fractions. In addition, *A. paniculata* also has properties other such as ethnobotanically used for treating snake bites, bug bites, diabetes, dysentery, fever, and malaria (Najila et al., 2002).

Table 1 Qualitative analysis of the phytochemicals in *Andrographis paniculata* extracts and its filtrates.

Bioactive constituents	Aqueous extract	Ethanolic extract	Aqueous filtrate	Alcoholic filtrate
Alkaloids	+	+	+	+
Glycosides	–	–	–	–
Cardiac glycosides	+	+	+	+
Phenols	+	+	+	+
Tannins	+	+	+	+
Phlobatannins	+	–	–	–
Hydrolysable tannins	+	+	–	–
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Saponins	+	+	+	+

+ = present, – = absent (Nagajothi et al., 2018)

Table 2 Bioactivities of compounds isolated from *Andrographis paniculata*

Bioactive Compound	Bioactivities
Andrographolide	Bioactivities
14-deoxyandrographolide	activation of NOS cyclase
	Vasorelaxant in vitro and in vivo.
Neoandrographolide	NO, PGE2, iNOS and COX-2 in activated
	macrophages CCl4, tBHP-induced
	hepatotoxicity (i.p 100 mg/kg, 3d)
14-deoxy-11,12-didehydroandrographolide	Muscle relaxant.
14-deoxy-14,15-didehydroandrographolide	NO release from endothelial cells.
	Cytotoxic activity and cell cycle arrest
	of tumor cells NF-κB-dependent
	trans-activation.
Andrograpanin	protein kinase or p38 MAPKs pathways
	chemokine SDF-1α induced
	chemotaxis in Jurkat and THP-1 cells.
Isoandrographolide	cell-differentiation-inducing
	activity proliferation of HL-60 cells.
14-acetylandrographolide	growth of leukeamia, ovarian, renal
	cancer cells.
19-O-acetylanhydroandrographolide	NF-κB-dependent trans-activation.

(Chao and Lin 2010)

Table 3 Percentage content of andrographolide, deoxyandrographolide and neoandrographolide in *A. paniculata*

Part Used	Yield of ethanol extract (%w/w)	Andrographolide (Mean±SD(%w/w))	Deoxyandrographolide (Mean±SD (%w/w))	Neoandrographolide (Mean±SD (%w/w))
Whole plant	7.83	3.54±0.25	1.05±0.22	0.83±0.19
Leaf	6.16	1.00±0.08	0.61±0.01	0.26±0.01
Stem	0.88	1.11±0.20	1.27±0.09	1.37±0.00
Root	3.25	0.16±0.04	0.68±0.10	0.70±0.14

(Cheung et al., 2001)

Table 4 The analysis of phytochemicals in the ethanolic extracts of *Andrographis paniculata.*

Phytochemical Tests	Resorts
Alkaloids (Mayer's reagent)	+
Carbohydrates & Glycosides (Molisch's test)	+
Sugar (Benedict's reagent)	+
Saponin (Foam test)	+
Protein (Millon's test)	+
Phytosterol (Libermann Burchard's test)	+
Phenolic compounds and tannin (Ferric chloride test)	+
Flavonoid (Alkaline reagent test)	+
Glycoside (Legal's test)	+

+ = present, – = absent (Rajalakshmi and Cathrine 2016)

Table 5 Pharmacological properties of various extracts of *Andrographis paniculata*

Chemicals	Pharmacological properties	References
Methanol extract restores	Plasma lipid peroxidation, ALT, AST activities in CCl4–treated rats (orally 1 g/kgBW, 14d).	Das et al., 2009
Ethanol extract	Serum anti–Salmonella *typhinurium* IgG levels	Xu et al., 2007
	IFN–γ in Con A–stimulated splenocytes of mice (Orally, 25 or 50 mg/kg BW, 14d)	
	antibody and the delayed–type hypersensitivity response (Orally 25 mg/kg, 7d) G0/G1 phase.	Puri et al., 1993
	mitochondrial CYP and expression of Bax in human leukemic HL–60 cells expression of EBV lytic proteins during the viral lytic cycle in P3HR1 cells	Lin et al., 2008
	fasting serum glucose in diabetic rats (orally 0.1, 0.2, and 0.4 g/BW, 14d) liver and kidney TBARS levels	Zhang et al., 2000
	liver GSH concentrations (orally 400 mg/kg BW, 14d)	
95% ethanol extract	RANTES secretion by human bronchial epithelial cells infected with influenza A virus H1N1.	Ko et al., 2006
80% ethanol extract	Hepatic GPX, GR, CAT, SOD: lipid peroxidation. (Orally 50, 100 mg/kg BW, 14d)	Singh et al., 2001
70% ethanol extract	CTL production through enhanced secretion of IL–2 and IFNγ by EL–4 T cells	Sheeja et al., 2007
	serum NO, VEGF and TIMP–1, angiogenesis in melanoma cell implanted mice (i.p. 10mg/d, 5d)	Sheeja et al., 2007
Aqueous extract	Protects nicotine–induced toxicity in brain (i.p. 250 mg/kg BW, 7d) nicotine induced DNA fragmentation in lymphocytes, lipid peroxidation, protein oxidation	Das et al., 2009
	systolic blood pressure of SHR and WKY rats (i.p. 0.7, 1.4, 2.8 g/kg BW)	Zhang and Tan 1996
	blood glucose in STZ–induced hyperglycaemic rats (50 mg/kg BW, 10d)	Husen et al., 2004
	hepatic CAT, SOD and GST activities in lymphoma. bearing mice (orally 10~30 m	Verma, 2008

2.2 Hybrid catfish

Figures 2 Hybrid catfish

The hybrid catfish is produced from a female Asian catfish, *Clarias microcephalus* and a male African catfish, *C. gariepinus*. The culture of this fish is rapidly gaining in popularity in Southeast Asia due to its rapid growth, resistance to disease, the possibility for high stocking density and excellent meat quality (Jantrarotai et al., 2007).

2.2.1 General characteristics of hybrid catfish

The appearance of hybrid catfish is like Asian mother catfish, *C. gariepinus* and African father catfish; its skin is yellowish. Dorsal fin base very long, usually with more than 30 rays, not preceded by a spine, separate or continuous with caudal fin; pectoral and pelvic fins variously absent in some species; caudal fin rounded; gill openings wide; usually four pairs of barbels; air-breathing labyrinthic organ arising from gill arches (Figure 2.2) Its body and tail are dotted with white dots, like those of the catfish *C. gariepinus* when they were small, but when fully grown, this dot will gradually disappear. The occipital skull is sharp with three notches. The head is large, and the tail has white dots at the age of about 3 weeks or more. The growth rate and appearance are more like African catfish. But the flesh is still yellow like that of the catfish, *C. gariepinus*. In addition, food habits are identical to African catfish. Therefore, it is in high demand on the market (Jantrarotai et al., 2007). Catfish is carnivorous and therefore require more protein in the feed than herbivorous species. However, it can be herbivorous when fed with ready mixed feed. In addition, the protein content of catfish feed should be 25-40%

depending on age and size of the fish. Approximately 30–50% of total proteins in the feed should be animal protein and the fat content should be 6–8% (Ng and Chen, 2002).

2.2.2 Distribution

Catfish are widely distributed in Southeast Asia, such as India, Myanmar, Thailand, Laos, Cambodia, the Philippines, Vietnam and Malaysia. Catfish can be found in canals, swamps, and marshes all over the world. It is a fish that lives in the water in general, including swamps with little water. This is because catfish is a fish that has a special organ for breathing. like snakehead fish. Therefore, they can survive in low oxygen condition Even in brackish waters, catfish can survive very well (Adan, 2000).

2.2.3 Habitat

Catfish are clearly different from other fish because they are without scales and long and slender bodies without no scale and with four pairs of tentacles on their lips. Tentacles are used to find feed because the eyes are so small. The antennae of the catfish have better sensory perception than the eyes. Catfish prefer living under the ground they have a nimble temperament and are able to stay on land for longer than other fish. Including being able to live in soil, mud, and water with low oxygen content for a long time because there is a special organ that helps in breathing. Their most prefervable ffod are meal. However, for the wihle ing ponds, catfish can be trained to eat plant–based foods and also to feed on the surface of water rather than on the grend (Adan, 2000).

2.2.4 Feeding

Catfish are carnivorous fish, When the catfish hatch into fry, the catfish will consume feed from the yolk sac attached to the front of the fry's abdomen for about 1–2 days.already It is necessary to find feed in the environment to eat. During this period, the catfish nursery needs to use feed for growth that contains a high amount of protein, such as boiled egg yolk, *Moina*, or mixed feed. Later, when the fish grow up, they can be released into the pond. The feed provided is rice bran, trash fish and pelleted feeds that float. In nature, catfish fry feeds the protozoa, rotifers and phytoplankton. The large catfish larvae feed on shrimp larvae, crab larvae, worms, and organic matter in the mud. In addition, they can be trained to eat either

submerged feed or floating pellets. which contains feed ingredients such as broken rice, mold, bean meal, fish meal, etc (Adan, 2000).

2.3 Hematology

2.3.1 Blood

Blood is a fluid plasma and cell-rich circulating tissue. It is made up of several types of cells (also known as corpuscles); these produced blood components account for around 45% of total blood volume. The remaining 55% is blood plasma, a yellow-colored fluid that serves as the blood's liquid medium. The usual pH of human arterial blood is 7.40 (normal range: 7.35–7.45), indicating a mild alkaline solution. Because blood accounts for around 7% of human body weight, the average adult has a blood volume of approximately 5 liters, of which 2.7–3 liters is plasma. The total surface area of all red cells in the human body would be approximately 2000 times that of the body's external surface (Alemu et al., 2006).

2.3.2 Function of blood

Blood has important transport, regulatory, and protective functions in the body.

1. Transportation

Blood transport oxygen from the lungs to the cells of the body and carbon dioxide from the cells to the lungs. It also carries nutrients from the gastrointestinal tract to the cells, heat and waste products away from cells and hormones form endocrine glands to other body cells.

2. Regulation

Blood regulates pH through buffers. It also adjusts body temperature through the heat-absorbing and coolant properties of its water content and its variable rate of flow through the skin, where excess heat can be lost to the environment. Blood osmotic pressure also influences the water content of cells, principally through dissolved ions and proteins.

3. Protection

The clotting mechanism protects against blood loss, and certain phagocytic white blood cells or specialized plasma proteins such as antibodies, interferon, and complement protect against foreign microbes and toxins (Klinken, 2002).

2.3.3 Platelets

These are tiny, non-nucleated round/oval cells/cell fragments that stain pale blue and have numerous pink granules. Their diameter ranges from 1-4μm. They are created in the bone marrow by the fragmentation of megakaryocytes, which are enormous, multinucleated cells. Their major role is to prevent blood loss from bleeding. When blood vessels are wounded, platelets quickly cling to the damaged vessel and join together to create a platelet block. During this process, the soluble blood coagulation factors are activated, resulting in the formation of an insoluble fibrin mesh surrounding the clumped platelets. This aids and strengthens the platelet plug, resulting in a blood clot that stops additional blood loss. Normal range: 150-400 x 10^3 /μl (Klinken, 2002).

2.3.4 Blood plasma

When the created components are extracted from blood, a straw-colored liquid known as plasma is left behind. Plasma contains approximately 91.5% water and 8.5% solutes, the majority of which (7%) are proteins by weight. Some proteins found in plasma are also present elsewhere in the body, but those found only in blood are referred to be plasma proteins. These proteins help to maintain normal blood osmotic pressure, which is essential for whole body fluid balance. The liver produces the majority of plasma proteins, including albumins (54%), globulins (38%), and fibrinogen (7%). Waste products like urea, uric acid, creatinine, ammonia, and bilirubin are among the other solutes found in plasma, as are nutrients, vitamins, regulatory chemicals such as enzymes and hormones, gases, and electrolytes (Klinken, 2002).

2.3.5 Red blood cell

They are the most numerous blood cells. They develop in the marrow of the bones that make up the axial skeleton. Mature red cells are nonnucleated and formed like flattened, bilaterally indented spheres, a shape known as a "biconcave disc" with a diameter of 7.0-8.0 μm and a thickness of 1.7-2.4 μm. Because stained smears only show flattened surfaces, the appearance is round, with a core pallor matching the indented areas.

The red blood cells contain the pigment hemoglobin, which can combine reversibly with oxygen. In the lungs, the hemoglobin in the red cells combines with oxygen and

releases it to the tissues of the body (where the oxygen tension is low) during its circulation. Carbon dioxide, a waste product of metabolism, is then absorbed from the tissues by the red blood cells and transported to the lungs to be exhaled. The red cell normally survives in the bloodstream for approximately 120 days, after which time it is removed by the phagocytes of the reticuloendothelial system, broken down, and some of its constituents are reutilized for the formation of new cells (Klinken, 2002).

2.3.6 White blood cell

They are a diverse collection of nucleated cells that are in charge of the body's defenses and are delivered by the blood to the various tissues where they perform physiologic functions, such as phagocytosis. WBCs are found in lower concentrations in normal blood than red blood cells (5.0–10.0 $10^3/\mu l$ in adults). They are made in bone marrow and lymphoid tissues (lymph nodes, lymph nodules, and the spleen) (Ashton, 2007).

2.4 Blood biochemical indices

The chemical constituent of the blood is a change in various substances in the serum. It can be used to indicate changes in bodily functions such as stress, infection, fasting, etc. Hematology determination and biochemistry in most aquatic animals are being studied in edible aquatic animals and endangered aquatic animals. There are many values of biochemical values that are commonly studied, for example:

2.4.1 Alanine amino transferase (ALT) and Aspartate amino transferase (AST)

ALT or alanine amino transferase and AST or aspartate amino transferase are not just enzymes that work on the connection between proteins and carbohydrates in metabolism, but also work as indicators of a change in the physiological status or stress and these enzymes were used to prove the damage in the liver and fish tissues (Al-Khshali and Hilali, 2019).

2.4.2 Creatinine

Creatinine is formed in muscles from creatine and creatine phosphate, at a rate which is about 1 to 2 % of the creatine–creatine phosphate body store per day (Hoberman et al., 1948). Creatine, is formed primarily in the liver, and is transported to the muscles, where its phosphorylated form, creatine phosphate, acts as a storage depot for muscle energy. However, The enzyme creatine phosphokinase catalyses the reaction between

creatine and creatine phosphate. Creatinine, a cyclic anhydride of creatine, is the metabolic endproduct of creatine metabolism (Al-Khshali and Hilali, 2019).

2.4.3 Alkaline phosphatases

Alkaline Phosphatase enzyme (ALP) takes a part in the process of active transport in the cell membrane, it is found on the simple epithelial cells and vertical cells in certain parts of the fish body in general. This enzyme has a role in the metabolism of carbohydrates, growth, cells differentiation, protein synthesis and the manufacturing and releasing of certain types of enzymes (Al-Khshali and Hilali, 2019).

2.4.4 Total protein

Plasma proteins have important physical properties, with albumin and globulin being involved in colloid osmotic pressure in the maintenance of body water balance. Globulins, which exist in the forms of alpha (α), beta (β), and gamma (γ), are involved in the formation of antibodies. Hormones and enzymes called fibrinogen help in blood clotting. All proteins in the plasma act as buffers, helping to regulate the pH level and cause blood viscosity (Belinskaia et al., 2021)

2.4.4.1 Albumin

Albumin is a family of globular proteins, the most common of which are serum albumins. All the albumin family proteins are water-soluble and moderately soluble in concentrated salt solutions. The essential qualities of albumin are those of an acidic, highly soluble and very stable protein, able to withstand temperatures of 60 °C for 10 hours. Albumins are commonly found in blood plasma and differ from other blood proteins because they are not glycosylated. Several other blood transport proteins are evolutionarily related to serum albumin, including alpha-fetoprotein, vitamin D-binding protein and afamin. This family is only found in vertebrates. Albumin serves in the transport of bilirubin, hormones, metals, vitamins, and drugs. It has an important role in fat metabolism by binding fatty acids and keeping them in a soluble form in plasma. This is one reason why hyperlipemia occurs in clinical situations of hypoalbuminemia. The binding of hormones by albumin regulates the amount of free hormone available at any time. Because of its negative charge, albumin is also able to furnish some of the anions needed to balance the cations of the plasma. Albumin is synthesized in the liver (Belinskaia et al., 2021).

2.4.4.2 Globulin

The globulin fraction contains hundreds of serum proteins, including carrier proteins, enzymes, complement, and immunoglobulins. The liver produces the majority of these, while plasma cells produce immunoglobulins. Electrophoresis separates globulins into four distinct categories. The four fractions are α1, α2 and are based on their migratory pattern between the anode and the cathode. Increases in the globulin fraction are mainly due to an increase in immunoglobulins, although there may be an increase in other proteins in pathologic situations with distinct electrophoretic patterns. Malnutrition and congenital immunological deficiency can produce a drop in total globulins due to reduced synthesis, whereas nephrotic disease can cause a decrease due to protein loss through the kidney (Belinskaia et al., 2021).

2.4.5 Glucose

Glucose is the carbohydrate normally found in mammalian blood and is referred to as the "blood sugar." With few exceptions, it is equally distributed in the water of the extracellular fluids; because of the lower water content of the red blood cells, serum or plasma glucose values average 10% to 15% greater than those of whole blood. Because of variable results that accompany differences in hematocrit values in the multiphasic system of whole blood, and because plasma is the transport medium, it appears desirable to determine glucose levels in serum or plasma (Belinskaia et al., 2021).

2.4.6 Cholesterol

Cholesterol is essential for the formation of bile acids, which allow you to be able to digest fats. Cholesterol is also utilized by the body to produce cell membranes. Everybody needs to have some cholesterol to be healthy, however it is when you have too much cholesterol that problems occur. High blood cholesterol is a major risk factor for coronary heart disease and for stroke. In addition, Cholesterol is a fatty substance, vital for good health. It helps form cell membranes, various hormones, bile and vitamin D. We get some cholesterol from our diet, but most is made in our liver (Murtola et al., 2011).

2.4.7 Triglyceride

Triglycerides (TGs) are essential fats (also called “lipids”) transported in our bloodstream with cholesterol. They are called triglycerides because each molecule contains

three fatty acids. TGs are the major source of energy used and stored by our bodies. They come from two sources—what we eat and what our liver makes. High blood TG levels can be genetic, or caused by diabetes, thyroid problems, kidney disease, or some medicines (Jomard and Osto 2020).

2.4.8 Low–density lipoprotein

Low–density lipoprotein (LDL) particles transport cholesterol and its esters in the bloodstream. LDL has a major role in the development of cardiovascular diseases, in particular atherosclerosis. In the initial stages of atherogenesis, LDL–derived lipids accumulate in the arterial intima, and high LDL levels have been shown to increase the risk of atherosclerosis (Murtola et al., 2011).

2.4.9 High Density Lipoproteins

High Density Lipoproteins (HDL) have long been considered as "good cholesterol," beneficial to the whole body and to cardio–vascular health. However, HDLs are complex particles that undergoes dynamic remodeling through interactions with various enzymes and tissues throughout their life cycle, making the complete understanding of its functions and roles more complicated than initially expected (Jomard and Osto 2020).

2.5. Fish immune system

The innate system is the original immune mechanism. It is distinguished by its non–specificity, which means that it does not rely on prior recognition of the invader's surface structures. It also benefits from being constitutive and reacting quickly, while simultaneously being able to be induced by outside molecules. However, genes encoding for the immunoglobulin superfamily have been discovered considerably earlier, demonstrating the broad potential of the innate immune system, even though the ability to synthesis several distinct antigen receptors is first observed in jawed fishes (Bayne, 2003).

2.5.1 Innate immunity

The innate immune response is the first mechanism for host defense found in all multicellular organisms. The innate immune system is more ancient than the acquired or adaptive immune response, and it has developed and evolved to protect the host from the surrounding environment in which a variety of toxins and infectious agents including bacteria, fungi, viruses and parasites are found (Janeway and Medzhitov 2002).

2.5.1.1 Lysozyme

Lysozyme is an important defence molecule of the innate immune system, which is important in mediating protection against microbial invasion. It is a mucolytic enzyme of leucocytic origin. Lysozyme is broadly dispersed in a wide range of animal secretions, including mucus and saliva, in various tissues, including blood, and in the cell vacuoles of plants. It is also present in bacteria, plants, invertebrates, and vertebrates. It splits the b (1–4) linkages between N–acetylmuramic acid and N–acetylglucosamine in the cell walls (peptidoglycan layers) of Gram–positive bacteria, thus preventing them from invading. The lysozyme does not directly harm gram–negative bacteria. Lysozyme works when complement and other enzymes break down the outer cell wall of Gram–negative bacteria, revealing the inner peptidoglycan layer of the bacteria. In addition to its antibacterial properties, it stimulates polymorphonuclear leucocytes and macrophages directly or indirectly through an opsoninic effect, which aids in phagocytosis. The enzyme also hydrolyzes glycol chitin, targets structures containing muramic acid, and has a limited ability to degrade chitin, a linear polymer of N–acetylglucosamine that is a significant component of the exoskeletons of several invertebrates and the cell walls of fungus. Furthermore, lysozyme is a food safe, antimicrobial enzyme that can also produce gels and flms (Bower et al., 2006)

2.5.2 Adaptive immunity

The innate immune system offers key mechanisms for pathogen detection and eradication. An expanded and improved repertoire of self– and nonself–antigen detection has been made possible by the development of adaptive immunity. Adaptive immunity is defined by a tightly regulated interaction between T and B lymphocytes and antigen–presenting cells, which promotes the development of immunologic memory, pathogen–specific immunologic effector pathways, and the maintenance of host immunological homeostasis. The series of lymphoid organs that make up the lymphatic system produce and activate lymphocytes. During development, gene segments are rearranged and combined to form genes that encode T and B cell antigen receptors. Rearrangement offers a vastly diverse spectrum of receptor specificities that can recognize elements of every potential pathogen. The development of immunologic memory is a crucial aspect of

adaptive immunity, in addition to specificity. Early on in an antigen–mediated response, sets of memory T and B cells are generated that are long–lived (pathogen). Memory cells are quickly activated when the same pathogen is contacted again, leading to a quicker and more powerful defensive response (Kurosaki et al., 2015).

2.5.2.1 Humoral

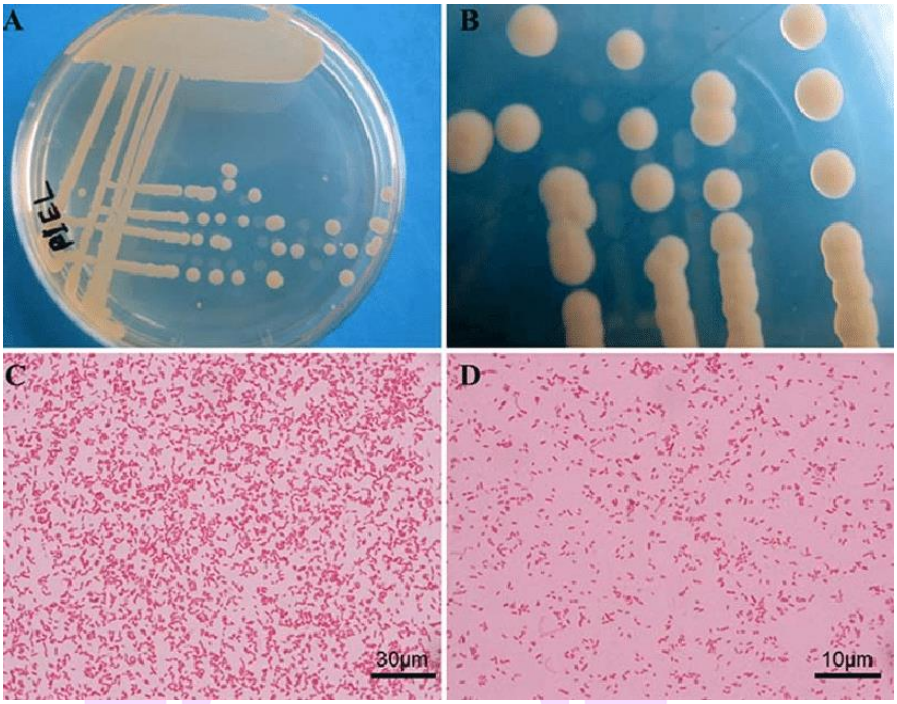
There is an array of soluble substances which have protective functions which inhibits the growth of microorganisms and neutralizes the enzymes on which pathogen depends. The classification of humoral parameters is commonly based on their pattern recognition specificities or effector functions (Kurosaki et al., 2015)

2.5.2.2 Memory B Cells

Postgerminal center memory B cells express Ig genes that have undergone isotype class switching and possess somatic mutations, and in humans they are distinguished by the presence of the marker CD27. The CD40–CD40L interaction contributes to directing GC B cells to mature into long–lived memory B cells. The exact life span of memory B cells is unknown. It has been postulated that these B cells either persist throughout the lifetime of the host or are renewed constantly through either nonspecific or antigen–specific stimulation.

Memory B cells circulate in a dormant condition throughout the body until a specific antigen is re–encountered, triggering a powerful secondary immune response. Memory cells react faster to antigen, need less antigen, and can even be triggered in the absence of antigen by soluble mediators such as IL–2 or IL–15, in part because the BCR is already localized to lipid rafts. Memory B cells, like naive B cells, consume antigen and express peptide–MHC class II fragments. Memory B cells expand and may develop into plasma cells after peptide antigen presentation to helper T cells (Kurosaki et al., 2015).

2.6 *Aeromonas hydrophila*



Figures 3 *Aeromonas hydrophila*

Nahar et al. (2016)

2.6.1 *Aeromonas hydrophila*

Aeromonas hydrophila is a heterotrophic, Gram–negative, rod–shaped bacteria that is mostly found in warm climates. These bacteria may be found in both fresh and salt water. It can live in both aerobic and anaerobic settings and digest substances such as gelatin and hemoglobin. In the 1950s, *A. hydrophila* was isolated from people and animals. It is the most well–known *Aeromonas* species (Figure 2.3). It is oxidase and indole–positive, and resistant to most popular antibiotics and freezing temperatures. *A. hydrophila* also has a symbiotic relationship as gut flora inside of certain leeches, such as *Hirudo medicinalis* (Sharon et al., 2003).

2.6.2 Genus characteristics of *Aeromonas* species

The genus *Aeromonas* has undergone a number of taxonomic and nomenclature revisions over the past 15 years. Although originally placed in the family Vibrionaceae, which also included the genera *Vibrio, Photobacterium*, and *Plesiomonas*, subsequent phylogenetic

investigations indicated that the genus *Aeromonas* is not closely related to vibrios but rather forms a monophyletic unit in the –3 subgroup of the class Proteobacteria. These conclusions necessitated the removal of *Aeromonas* from the family Vibrionaceae and transfer to a new family, the Aeromonadaceae. Similarly, only five species of *Aeromonas* were recognized 15 years ago, three of which *(A. hydrophila, A. sobria*, and *A. caviae*) existed as phenospecies, that is, a named species containing multiple DNA groups, the members of which could not be distinguished from one another by simple biochemical characteristics. (Sharon et al., 2003).

2.6.3 Natural Habitat for *A. hydrophila*

Many of the species in the three genera are free–living saprophytes although some are associated with reptiles, fish and animals. *Aeromonas* spp. are widespread in freshwater, sewage and soil. Their numbers rise with the amount of organic matter present. *A. hydrophila* is part of the normal flora of freshwater fish and is commonly present in fish ponds and tanks. Animals can be faecal carriers of *Aeromonas* spp. (Sharon et al., 2003).

2.6.4 Structure of *A. hydrophila*

A. hydrophila bacteria are Gram–negative, straight rods with rounded ends (bacilli to coccibacilli shape) usually from 0.3 to 1.0 μm in width and 1.0 to 3.0 μm in length. They can grow at temperatures as low as 4 °C. These bacteria are motile by a polar flagellum (Sharon et al., 2003).

2.6.5 Fish and amphibians

A. hydrophila is a waterborne, gram–negative bacillus that is a commensal inhabitant of the gastrointestinal tract of clinicallyhealthy frogs. Stress and consequent immunomodulation predispose amphibians to *A. hydrophila* colonization and clinical disease. *Aeromoniasis* is a communicable disease of teleost fishes, amphibians, and reptiles. The skin and visceral organs are common sites of *A. hydrophila* colonization in amphibians, and clinical presentation may include cutaneous petechiation and ulceration, lethargy, anorexia, edema, and neurologic signs. *A. hydrophila* infection in the frog we describe here was determined by bacterial culture of skin and liver and was associated with cutaneous ulceration (Densmore and Green 2007).

2.7 Related research

Rattanachaikunsopon and Phumkhachorn (2009) reported that *A. paniculata* supplementation could increase the survival rate of *Oreochromis niloticus* infected with *Streptococcus agalactiae* and had no effect on fish on the ratio 4:36 and 5:35 (wt/wt). Moreover, Pandey and Mandal (2010) study about the pharmacological properties of *A. paniculata* showed that of medicines for number of ailments related to digestion, hepatoprotection, hypoglycemic, anti–bacterial, analgesic, anti–inflammatory, vermicidal and antipyretic. In addition, the average andrographolide content varied from 1.07 to 2.24 % in dried leaves. Furthermore, Basha et al. (2013) indicated that Andrographolide has effects on growth performance, non–specific immune parameters and resistance against *A.hydrophila* in *Labeo rohita* at a concentration 0.1%. Beyond, Palanikani et al. (2018) showed that *A. paniculata* supplementation can be against the pathogens *A.hydrophila* and *A. veronii.* in Indian carp. In addition, can increased hemoglobin, erythrocyte, leukocyte, phagocytic index and survival rate at a concentration 50 µl. Moreover, Roongkamnertwongsa and Roongkamnertwongsa (2012) reported that supplementation with *A. paniculata* crude extract in the diet of seabass *(Lates calcarifer*) at 5 g/kg can improve the number of red blood cell, lysozyme activity, superoxide anion and phagocytosis. In addition, the mortality of post–bacterially challenged fish was lower in the fish–fed crude extract diet. Other than that, Sheeba, (2012) showed that *Catla Catla* increased red blood cells, white blood cells, hemoglobin and serum protein. Furthermore, *A. paniculata* extract can improve nonspecific immunity and resistance to *A. hydrophila*. Except, Palanikani et al. (2020) indicated that *A. paniculata* extract at a dose of 50 µl can increase the levels of hemoglobin, erythrocyte and leucocyte, along with the phagocytic index. The extracts also had a significant impact on modifying the anatomy and swimming pattern of infected fish, post treatment with the extracts, In addition, This herb has efficacy against *A. hydrophila* in *Labeo rohita.* Furthermore, Charoendat et al. (2016) indicated that *A. paniculata* extract cannot promote growth performance of Pacific white shrimp (*Litopenaeus vannamei* Boone), but it can be used for supporting shrimp health because application of extract as a dietary supplement at a concentration level of 60

ppm can increase the survival rate of shrimp infected with *Vibrio harveyi*. Other than, Shi et al. (2020) reported that the diets supplemented with Andrographolide at concentrations (75 and 150 mg/kg) improved growth performance, enhanced antioxidant capacity and regulated the intestinal physical barrier and microbiota of *M. albus.* In addition, dietary supplementation of andrographolide upregulated anti–inflammatory cytokines and downregulated proinflammatory cytokines. Besides, Maiti et al. (2021) demonstrated that *A. paniculata* leaf extract at a concentration 2% has effects on growth, immunomodulation, and disease outbreak against *A. hydrophila* and has a positive effect on hematobiochemical parameters and immune response in *Pangasianodon hypophthalmus*. In addition, Phommanivong and Doolgindachbaporn (2013) reported that *Moringa oleifera* supplementation 15% can improve growth and survival rate of hybrid catfish (*Clarias macrocephalus x C. gariepinus*). Besides, Panase et al. (2018) showed that *Houttuynia cordata Thunb.* leaf extract at 70–ml/kg can improve average daily growth (ADG), specific growth rate (SGR), feed efficiency (FE) and protein efficiency ratio (PER), as well as the lowest feed conversion rate (FCR). In addition, this concentration can increase the total red blood cell (RBC), total white blood cell (WBC) and hemocrit percentage (Htc) of hybrid catfish (*Clarias macrocephalus × Clarias gariepinus*). Moreover, Panase et al. (2018) indicated that hybrid catfish, *Clarias macrocephalus × C. gariepinus* were promote growth performance, hematological and some organosomatic indices by *Euphorbia hirta* plant leaf extract at 300 mg/kg. In addition, Munglue et al. (2019) reported that hybrid catfish (*Clarias macrocephalus × C. gariepinus*) fed diet supplemented with *Limnophila aromatica* extract showed an increase in weight gain, specific growth rate and feed utilization efficiency when compared to the control diet and that the extract could treat inflammation in the intestine of fish. Other than, Sheikhlar et al. (2017) found that methanolic *Euphorbia hirta* extract has antimicrobial activity against the bacterium *Aeromonas hydrophila* but has no effect on growth, feeding efficiencies, hypatosomatic index (HSI) and plasma biochemical parameters in African catfish (*Clarias gariepinus*). Moreover, Khunchalee and Munglue (2020) Cardamonin Enriched diets affected enhanced growth parameters and feed utilization efficiency. but hematological indices, including hemoglobin, hematocrit, white blood cell, red blood cell, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, did

not differ among the treatments (P>0.05). Meanwhile, albumin levels of the tested fish were significantly increased compared with the control (P<0.05) in hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*). Furthermore, Gabrilel et al. (2019) showed that African catfish (*Clarias gariepinus*) had increased growth parameters, i.e., weight gain, absolute growth rate, specific growth rate, protein efficiency ratio and decreased feed conversion ratio. In addition, improved hemato–biochemical indices can improve their survival rate by Aloe vera extract at 4%. In addition, Sheikhlar et al. (2014) indicated that *Morus alba* (white mulberry) foliage extract at a level of 7 g/kg can inhibit *Aeromonas hydrophila* and improve red blood cell (RBC), hemoglobin, hematocrit, globulin, albumin, total protein and reduce mortality rate in African catfish (*Clarias gariepinus*). Beyond, Dada and Sonibare (2015) reported that dietary supplementation with chromolaena odorata leaf extract powder improved growth rate, feed utilization, white blood cells and survival of *Clarias gariepinus*. Furthermore, Kartikaningsih et al. (2020) reported that Characteristics of *A. hydrophila* infected Catfish (*Clarias* sp.*)* had clinical symptoms, such as swelling on stomach and intestines were black on days 5 to 7, in the skin injured on day two and hemorrhagic on day 6–7. The organoleptic test indicated that the quality of the mucus, texture, and odor was reduced. Furthermore, the results of the histological test reported stretching of the muscle. Moreover, Zheng et al. (2009) showed that *Origanum heracleoticum* extract can stimulate growth promoter, increase antioxidant activity, enhance muscle protein sedimentation and also improve disease resistance to pathogens when added to channel catfish (Ictalurus punctatus) feed.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Equipments for experiment

3.1.1 Equipments for fish culture

Juvenile hybrid catfish

Pellet Food Fish (protein 30%)

A. paniculata (Stem and Leaf)

Centrifuge Dynamica (Velocity 18R)

Pocky spray

Hemocytometer

Agar Powder

Cloth gloves

lectric pan

plastic basin

Hand towel

landing net

Digital scales

Poly Bag with Zipper

Foam box

K3EDTA tube

Capillary Tube

Eppendorf (1.5 ml)

Syringe and hypodermic needle 25 G X 1

Hematocrit Centrifuge (HC–12C)

microcentrifuge tube racks

Test tube rack Stainless

Micropipette (100–1000 µl)

Eppendorf tube (1.5 ml)

Microscope

Cover slide

Hand count

Phone

Calculate

Scalpel

Forceps

Cage

Pen

Ice

3.1.2 Equipments for herbal extraction

Filter paper (Whatman 110 mm)

Rotary Evaporator (N–1000 EYELA)

Cross beater mill (SK 300)

Round bottom flask

Weighing scale

Evaporating disk

Hand towel

Tissue paper

Refrigerator

Plant herb

Ethanol 95%

Ascot glass jar

Hot air oven

Glass funnel

Suction flask

Buchner funnel

Glass rod

Dropper

Beaker

Cylinder

Glove

Spatula

Knife

3.1.3 Laboratory equipments

Aeromonas hydrophila

Micrococcus lysodeikticus

Cell culture dishes

Freezer (SANDEN SNH-0205)

Latex gloves

Analytical Balance (Ohaus)

Weighing Paper

Autoclave (HVA-85)

Aluminum tray

Aluminum foil

Hand Towel

Incubator shaker (VS-8480SFN)

Duran Bottle (500 and 1000 cm)

Eppendorf Centrifuge (5810R)

Incubator (LBI-150E)

Centrifuge tube (50 cm)

TSA (Tryptic Soy Agar)

laminar air flow (AHC-4D1)

Vortex Genie 2

Alcohol Burner

DI water

Hot plate

Forceps

Loop

3.1.4 Chemicals

$NaCl_2$ (0.1%)

Magnesium ribbon

HCl

KL

Di water

Iodine

Mercury

Chloride

$HgCl_2$

CH_4CooH

Ferric Chloride

$CHCl_3$

Acetic Anhydride

Kedde reagent

NaCL

KCL

Na_2HPO_4

K_2HPO_4

Formaldehyde

Trisodium citrate

Glacial acetic acid

Gentian Violet

3.2 Preparation of herbal extract

Fresh leaf and Stem of *A. paniculata* plants were obtained from the University of Phayao, Thailand. Fresh *A. paniculata* was gathered and rinsed thoroughly in water before air drying. The plants were dried for 96 hours in an oven at 4–45°C. The dried herbs were finely ground (0.9 mm) and the plant powder was soaked in ethanol. 250 g of plant powder was combined with 1000 ml of 95 percent ethanol and stored at room temperature for 96 hours. The mixture was then filtered through (Whatman filter paper

No. 1), before being evaporated to get rid of solvent (below 40°C) using a rotary evaporator. The extract was stored in a test tube and wrapped in foil and then was stored in the refrigerator.

3.3 Phytochemical screening

1. Test for flavonoids

The test solution of the extracts was dissolved in 95% ethanol. To this, a small piece of magnesium foil metal was added; this was followed by 3–5 drops of the concentrated HCl. The intense cherry red color indicated the presence of flavonoids (Abdalla et al., 2020).

2. Test for alkaloids

2.1 Wagner's test

A few drops of 0.44 mol/L Wagner's reagent (solution of iodine in potassium iodide, 2 g of iodine and 6 g of potassium iodide were dissolved in 100 mL distilled water) were added to 2 mL of each filtrate along the side of the test tube; a positive test, demonstrating the presence of alkaloids, was indicated by the formation of reddish–brown precipitate (Abdalla et al., 2020).

2.2 Mayer's test

One or two drops of 0.35 mol/L Mayer's reagent (potassiummercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 mL distilled H_2O) were added to 2 mL of each filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a white creamy precipitate (Kokate, 2001).

2.3 Dragendorff's test

Dragendorff's reagent was made of two solutions. Solution A contained 1.7 g basic bismuth nitrate in 100 mL water/ glacial acetic acid (80 mL water and 20 mL glacial acetic acid in a 4:1 ratio), and solution B contained 40.0 g potassium iodide in 100 mL of water. Both solutions were mixed in the following manner to produce 100 mL Dragendorff's reagent (5 mL solution A + 5 mL solution B + 20 mL glacial acetic acid + 70 mL water). Dragendorff's reagent at 0.136 mol/L was added (1–2 mL) to each of the

2 mL of filtrate solutions. The formation of an orange–red precipitate indicated the presence of alkaloids (Kokate, 2001).

2.4 Molisch's test

Molisch's test for alhaleids: Few drops of Molisch's reagent were added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then 5 of distilled water. The formation of a red or dull violet color at the interphase of the two layers was a positive test (Kokate et. Al., 2001).

3. Test for anthraquinone (Borntrager's test)

The reaction mixture contained 6 N freshly prepared HCl (3 mL) and the extract then heat for 10 min, 3 mL dichloromethane was added and shaken for 5 min. The extract was filtered, and the filtrate was shaken with an equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone (Rauf et al., 2013).

4. Test for coumarins

In a test tube, 1 g of each of the extracts was placed and covered with filter paper moistened with 20% NaOH, then heated on a water bath for 10 minutes. The filter paper was examined under UV light; yellow fluorescence indicated the presence of coumarins (Rauf et al., 2013).

5. Test for tannins (Ferric chloride Test)

The 95% ethanolic extract was treated with a 1% ferric chloride test solution. The resultant color was investigated. A blue color indicated the presence of hydrolyzable tannin. Into 10 mL of freshly prepared KOH in a beaker, 0.5 g of the extract was added and shaken to dissolve. A dirty precipitate observed indicates the presence of tannin (Rauf et al., 2013).

6. Test for saponins

0.2g of each extract was shaken with 5 ml of distilled water and heated to boiling. Frothing (appearance of creamy mist of small bubbles) shows the presence of saponins (Rauf et al., 2013).

7. Test for terpenoids

Five ml of each extract was mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish–brown coloration of the interface was formed to show positive results for the presence of terpenoids (Abdalla et al., 2020).

8. Test for Steroids

The dichloromethane extract was evaporated to dryness and the residue was dissolved with acetic anhydride. The concentrated H_2SO_4 was then added through the sides of the evaporating disc. A reddish–brown coloration of the interface indicated the presence of terpenoids. A blue coloration of the interface indicated the presence of steroids (Chandra et al., 2014).

9. Test for cardiac glycosides

9.1 Kedde's Reagent**:** The test solution of the extracts was dissolved in 95% ethanol. The extract was evaporated to dryness, and Kedde's Reagent A (2% 3,5–dinitrobenzoic in ethanol) and B (5% KOH in ethanol) were added to the residue. Purple or reddish–violet color reveals the presence of an unsaturated lactone ring (Chandra et al., 2014).

9.2 Keller–Kiliani Test: The dichloromethane extract was evaporated to dryness, and residue was dissolved in 3 mL of glacial acetic acid, followed by an addition of a few drops of $FeCl_3$ solution. The resultant solution was transferred to a test tube containing 2 mL of the concentrated H_2SO^4. The reddish–brown layer was formed, which turns bluish green after standing due to the presence of deoxy sugar (Chandra et al., 2014).

9.3 Molisch tests: Molisch's Test: To 1 ml of extract, 2 drops of Molisch's regent were added in a test tube and 2 ml of concentrate H2SO4 was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of glycosides (Kokate et. al., 2001).

3.4 Experimental fish and the conditions for acclimatization

The procedures for this research, concerning animal care and experimentation, were approved by the Committee for the Institution of Animal Care at the University of Phayao, Thailand (ID: 1–030–65). Hybrid catfish *(Clarias macrocephalus × C. gariepinus)* were bought

from a fish farm in Phayao Province Thailand. The experimental fish were transferred to the lab and acclimatized for two weeks in a cement pond (2 × 3 × 0.5 m) containing 3000 L of water under a natural photoperiod, with a continuous aeration and water recirculation system employed weekly. During the acclimation, the following water quality parameters were observed: the temperature was kept constant at 26.3 ± 1.23 °C, the dissolved oxygen (DO) concentration was kept constant at 7.7 ± 0.52 mg/l, and the pH was kept constant at 7.6 ± 0.35 throughout the experiment. The experimental fish were fed with commercial fish feed at a rate of 7% of their body weight per day (30% crude protein) twice daily (08.00; 17.00) without *A. paniculata* extract.

3.5 Supplemented diet preparation

Throughout the experiment, a commercially available fish diet containing 30% crude protein (fat 4%, moisture 12% and fiber 8%) was employed. Treatment 1 was not sprayed with *A. paniculata* extract; treatment 2, 3 and 4 were sprayed and completely combined in a drum mixer in the ratio of 200, 400 and 600 mg/kg, respectively. For all groups, the pellets (2 mm in diameter) were coated with a 4 percent agar solution at a concentration of 10 ml/kg and air dried again. They were then stored at room temperature in separate sterile containers for seven days, and the feed was quickly devoured by the experimental fish.

3.6 Experimental design

Healthy fish with an average body weight of 10.5 ± 0.44 g and a mean total length of 5.08 ± 0.52 cm was placed in 12 net cages (1 x 2 x 0.8 m; mesh size, 2.5 mm^2) in four triplicate groups after acclimatization. All groups were placed in the same earthen pond, with a stocking density of 30 fish per net cage. All groups were fed a diet tailored to their needs at 7% of their body weight twice a day. Water quality was measured even though all net cages were placed in the same earthen pond. The observed water quality parameters were as follows: water temperature fluctuated between 27.8–29.6°C, dissolved oxygen 6.3–7.82 mg/L, pH 7.23–8.79, and total dissolved solids 0.21 0.52 g/L.

3.7 Growth performance and survival evaluation

At 15–day intervals, all the fish in each net cage were weighed to adjust feed volume, and evaluate growth parameters such as weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed conversion rate (FCR), feed efficiency (FE), protein efficiency ratio (PER) and survival rate (SR), which were determined using the following equations (Bagenal, 1978)

WG = final weight (g) – initial weight (g)

ADG = [{final weight (g) – initial weight (g)}{Adler, 1997 #2}/ experimental days

SGR = [{Ln final weight (g) – Ln initial weight (g)}/ experimental days] × 100

FCR = total feed fed (g) / weight gain (g)

FE = weight gain (g) / total feed (g)

PER = wet weight gain (g) / crude protein fed

SR = [number of survived fish / initial number of fish] × 100

3.8 Study of hematological indices

Nine fish/groups (three fish/replications) were chosen at random, and they were given a 3–minute anesthesia with MS–222 1: 4000 in dechlorinated water (Harikrishnan et al. 2010). On days 30, 60, and 90 of the experimental periods, blood samples were taken from the caudal vein (0.8 ml) using syringes with 26G needles that contained blood in K3 EDTA tubes. A blood sample was diluted 1:300 in a 0.85% NaCl solution to determine the total red blood cell levels (RBC 10^9 mm^3), and the hematocrit percentage (%Hct) of the blood samples was measured using the microcentrifuge method. A blood sample was diluted 1:300 in a 2% solution of acetic acid to assess the total white blood cell level (WBC 10^3 mm^3), RBC and WBC were manually counted in a Neubauer chamber (Rehulka, 2003).

3.9 Study of serum biochemicals indices

Serum biochemistries were investigated on days 30, 60 and 90 of the feeding trial. Following the evaluation of growth indices and survival, nine fish from each group (3 fish each replication) were chosen at random for blood collection. Non–heparinized syringes were used to draw blood samples (0.8 mL/ fish) from the fishes' caudal veins. Blood samples were put into micro–centrifuge tubes and allowed to clot at room

temperature for 4 hours before being used to collect serum. After this, they were put into a centrifuge at 5000 rpm for 15 minutes at 25 °C, and the supernatants were kept in sterile serum tubes at −20 °C until required (not for longer than 7 days). The serum samples were frozen and sent to the Chiang Mai Veterinary Laboratory Centre Limited Partnership in Chiang Mai, Thailand, for analysis. Serum indices, activities of the alanine transaminase (ALT), aspartate transaminase (AST), creatinine, alkaline phosphatase, triglycerides, glucose, cholesterol, total protein, albumin, globulin, low density lipoprotein, high−density lipoprotein were evaluated using an analytical chemistry analyzer (P400 and PC400, HORIBA, Japan)

3.10 Study of serum lysozyme activity

Lysozyme activity was checked every 30 days, nine fish from each group were randomly selected for serum preparation. Lysozyme activity was measured using the turbidimetric method (Koskela et al., 2004) with some modifications. The maximum amount of activity was detected in a 0.05 M phosphate buffer, pH 6.0, in which the substrate, lyophilized *Micrococcus lysodeikticus*, was suspended (3.0 mg/mL). 25 μL serum from each sample was then added to 100 μL of the bacterial suspension. The absorbance rate was measured at 450 nm (Multimode Reader LB 942) at intervals of the 30s (total measuring time 3 min) at 25 °C. The results were expressed as unit/mL, which was computed using one unit of lysozyme activity, and was defined as 0.001 per min of the absorbance rate and was then reduced.

3.11 Study of organosomatic indices

At the end of the experiment, three fish from each net cage (replicate) were selected randomly and anesthetized with MS−222 1:4000 in dechlorinated water for 3 min (Harikrishnan et al., 2010). The visceral organs were separated so that the liver, spleen, kidney and intestine could be used to calculate the hepatosomatic index (HSI,%), spleenosomatic index (SSI %), kidney (KI %) and intestinosomatic index (ISI%), respectively. Calculations were computed as follows (Ronald and Bruce (1990); Hadidi et al., 2008):

HSI = [liver weight (g)/body weight (g)] × 100.

SSI = [spleen weight (g)/body weight (g)] × 100.

KI = [kidney weight (g)/body weight (g)] × 100.

ISI = [intestine weight (g)/body weight (g)] × 100.

3.12 Bacterial culture and determination of LD_{50}

Aeromonas hydrophila was procured from the Faculty of Fisheries, Kasetsart University (Thailand), and bacterial preparation for the challenge test on the *A. hydrophila* was done by culturing the sample at 25 °C in tryptic soy broth (TSB) for 24 h. The culture was centrifuged at 3,000 rpm for 10 minutes. The supernatants were discarded, and the suspension was held in phosphate buffered saline (PBS, pH 7). The bacterial suspension was adjusted to an optical density (OD) of 600 nm to correspond to approximately 10^9 CFU/ml for inducing fish infection as follows by (Wangkahart, 2018).

The virulence and pathogenicity were tested using LD_{50} before the challenge test on *Clarias macrocephalus* × *C. gariepinus*, 10^9 CFU/mL were found. To study the bacterial resistance to *A. hydrophila*, in the *Clarias macrocephalus* × *C. gariepinus*, 120 fish (10 fish per replication) from each group, were used. After 90 days of feeding trials, the four groups were injected intraperitoneally with 1.5 mL of 10^9 CFU/mL. The cumulative mortality rates (%) for each group were monitored for 14 days after the bacterial injection.

3.13 Study of hematological and serum lysozyme activity after *A. hydrophila* challenged test

Hematological indices such as red blood cell (RBC), white blood cell (WBC), hematocrit (Htc.) and serum lysozyme activity were determined in the surviving fish after day 14, post–injection. Three fish from each group were randomly selected for blood and serum preparation by the method performed above mentioned.

3.14 Statistical analysis

The normality and homogeneity of variance were tested before the analysis. Statistical analysis of data involved a one–way analysis of variance (ANOVA) followed by a Tukey's test at a significance level of 95 % ($p < 0.05$). Statistical analyses were done using SPSS software version 25 for windows (SPSS Inc., Chicago, USA). All data were presented as mean ± SD.

CHAPTER 4

RESULTS

4.1 Phytochemical analysis

The results revealed that phytochemical constituents such as coumarins, tannins, terpenoids, lactones, steroids, and sugars were found in the ethanolic extract of *A. paniculata* but flavonoids, alkaloids, saponins, cardiac glycosides, cyanogenic glycosides, and anthraquinones were not found in this study (Table 6).

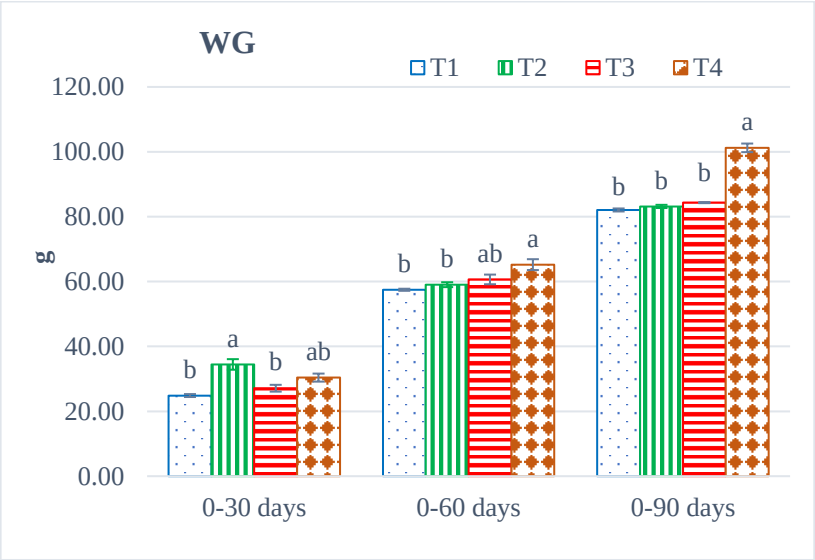
Table 6 Phytochemical constituents of 95 % ethanol extract from *A. paniculata.*

Phytochemicals	Results
Flavonoids	–
Alkaloids	–
Anthraquinones	–
Coumarins	+
Tannins/ Phenolics	+
Saponins	–
Terpenoids	+
Lactones (5–membered unsaturated)	+
Steroids	+
Cardiac glycosides	–
Cyanogenic glycosides	–
Sugars/Carbohydrates	+

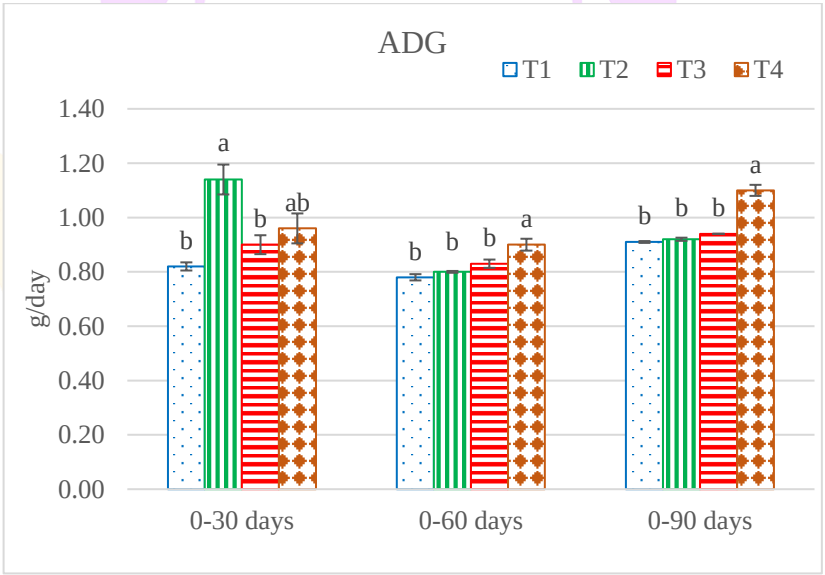
+ present, – absent.

4.2 Growth indices and survival rate

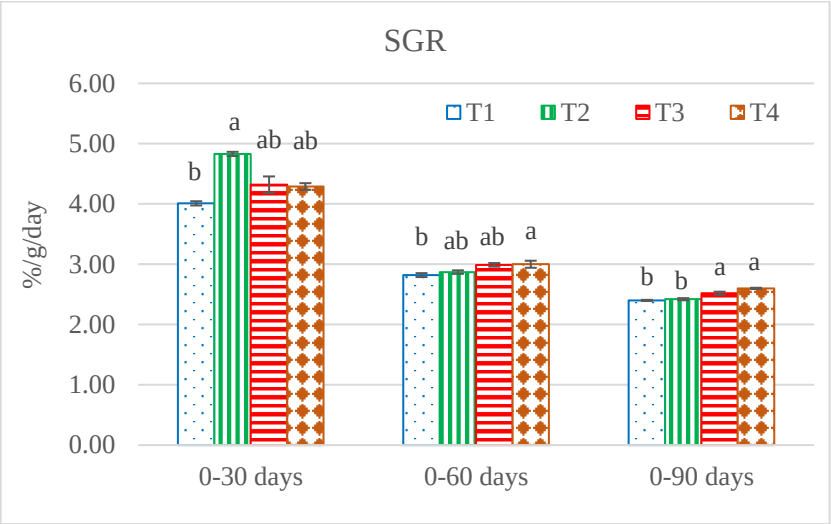
The growth indices of *C. macrocephalus* × *C. gariepinus* showed that weight gain (WG) was significantly different ($p < 0.05$). At day 30 the highest WG was found in group fed 0.2 g/kg (T2) when compared to control group ($p < 0.05$), but on the 60 and 90 days, the group fed 0.6 g/kg (T4) was found the highest WG compared to other dietary groups (Fig. 4). The average daily gain (ADG) was significantly different ($p < 0.05$) from days 30, 60 and 90. The group fed T2 had the highest ADG compared to the other groups on day 30; the group fed T4 had the highest ADG compared to the control group on days 60 and 90 (Fig. 5). The specific growth rate (SGR) had a significant difference on day 30 of the experiment, the highest value and the lowest value were found in groups fed T4 and T1, respectively, and there was also a significant difference on days 60 and 90. The higher values were found in groups fed T4 and T3, but the lower value was found in group fed T1 (Fig. 6). The survival rate was not different ($p > 0.05$) throughout the experimental period (Fig. 7). The feed conversion rate (FCR) was significantly different on days 60 and 90 of the experiment. On day 60, the highest FCR was found in the group fed T2 and control group (T1), but on day 90, a lower level was found in the group fed T4 (Fig. 8). While feed efficiency (FE) showed a significant difference on days 60 and 90 of the experiment, on both days 60 and 90, the highest value was found in the group fed T4 (Fig. 9). Protein efficiency ratio (PER) showed a significant difference on days 60 and 90; at day 60, the fed T4 had the highest value, but on day 90, the highest and lowest values were found in the group T4 and T1, respectively (Fig. 10).



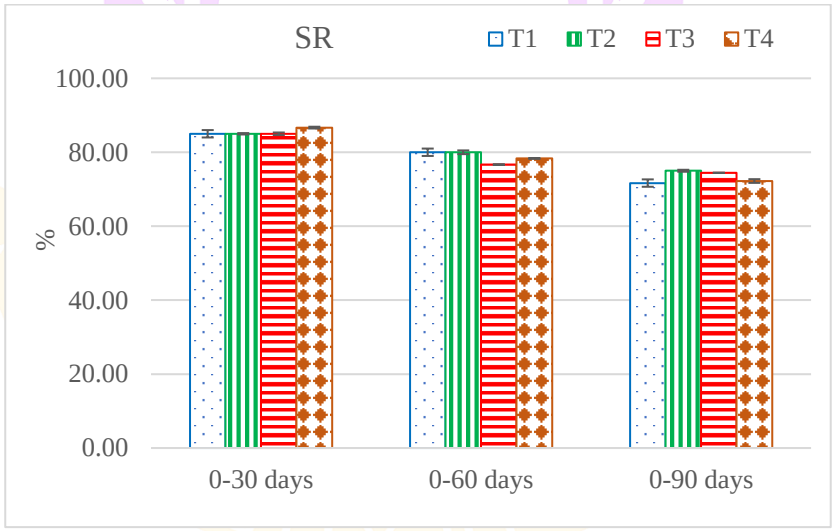
Figures 4 Weight gain of *Clarias macrocephalus X C. gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



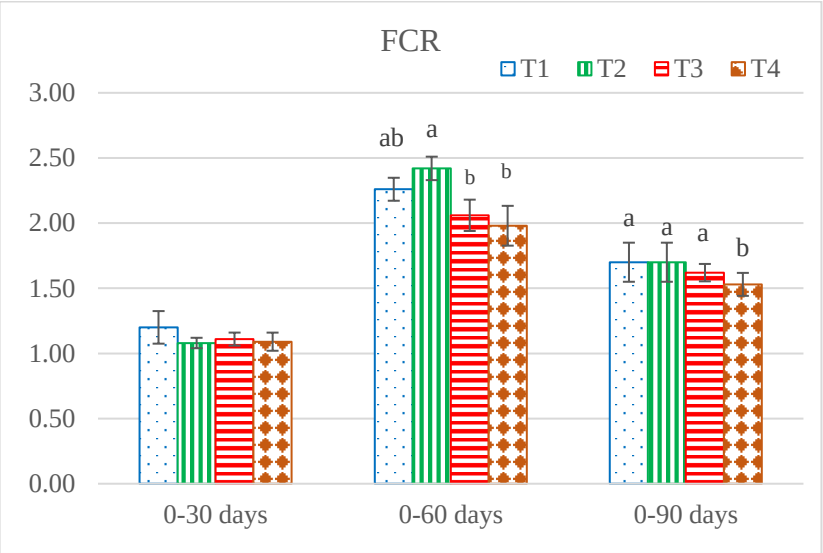
Figures 5 Average daily gain of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



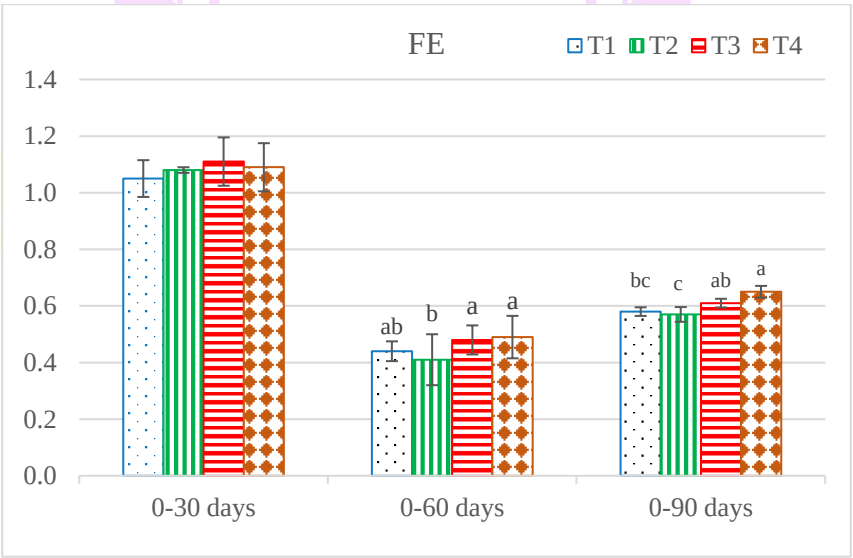
Figures 6 Specific growth rate of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



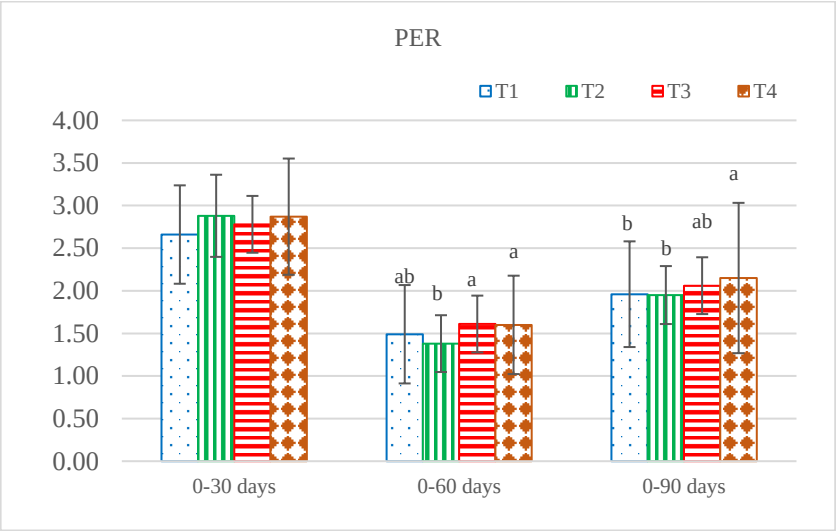
Figures 7 Survival rate of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate no significant differences ($p > 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



Figures 8 Feed conversion rate of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



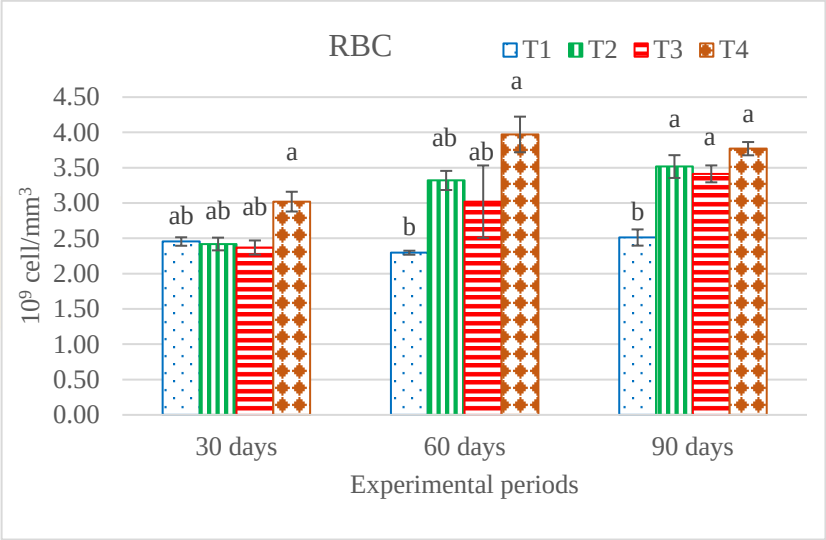
Figures 9 Feed efficiency of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).



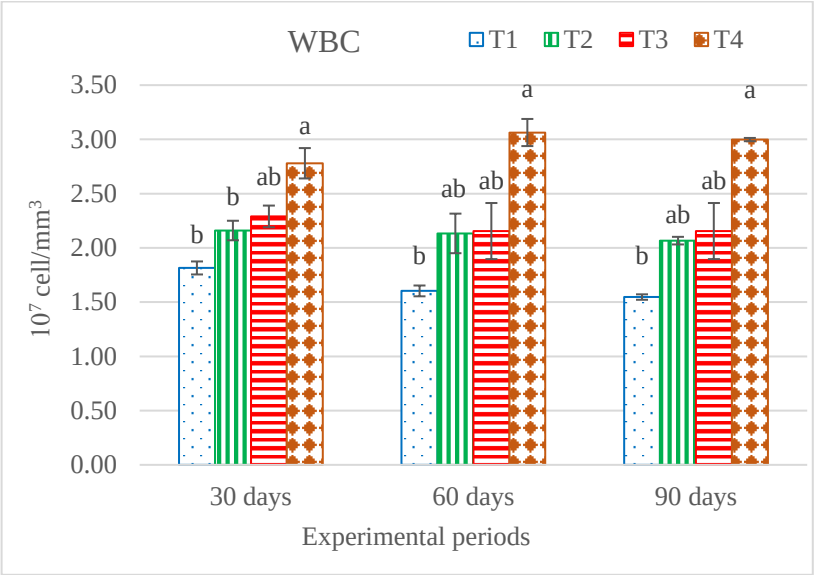
Figures 10 Protein efficiency ratio of *Clarias macrocephalus X Clarias gariepinus*, which were fed different levels of *A. paniculata*. Different letters indicate significant differences ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g/kg), T3 (0.4 g/kg) and T4 (0.6 g/kg).

4.3 Hematological indices

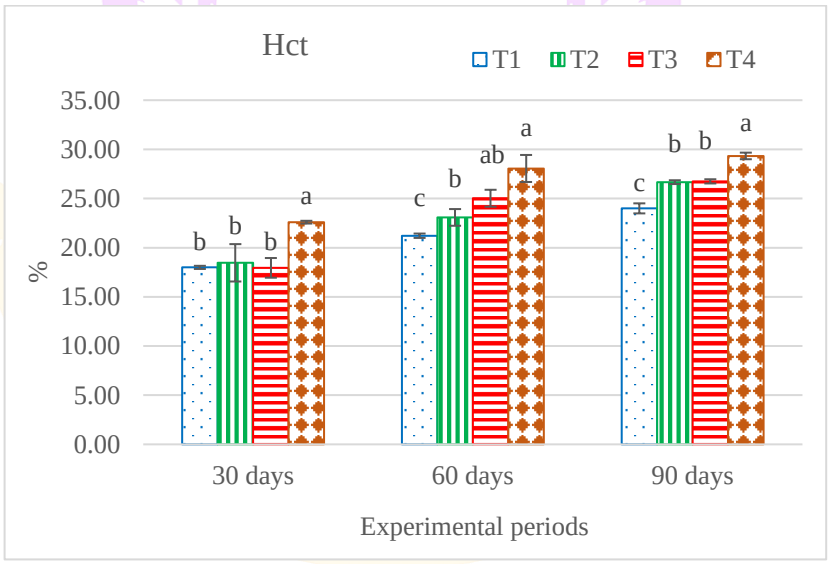
In the first sampling on day 30, RBC (total red blood) in T4 exhibited a significantly ($P < 0.05$) higher value (3.02×10^9 /mm^3) than the other treatment groups. The values ($3.97 \times 10^9/mm^3$ on day 60, $3.77 \times 10^9/mm^3$ on day 90) of RBC in T4 were found to be significantly ($P < 0.05$) higher in consecutive samplings (days 30, 60, and 90) as compared to the values of other treatments (Fig 11). WBC (Total white blood cell) of T2, T3, and T4 exhibited significantly ($P < 0.05$) higher values than that in the control (T1) in all the sampling days. Further, WBC values in T4 were significantly ($P < 0.05$) higher compared to T1, T2 and T3 groups in all the sampling days (Fig 9). Hematocrit percentage (Htc), values (22.60 % on day 30, 28.05 % on day 60 and 28.66 % on day 90) in T4 were found to be increasing in consecutive samplings and significantly ($P < 0.05$) highest as compared to the values of other treatments (Fig 12–13).



Figures 11 Total red blood cells, (RBC) which were fed different levels of *A. paniculata* extract for 30, 60, and 90 days: Significant differences ($p < 0.05$) is indicated by different letters.



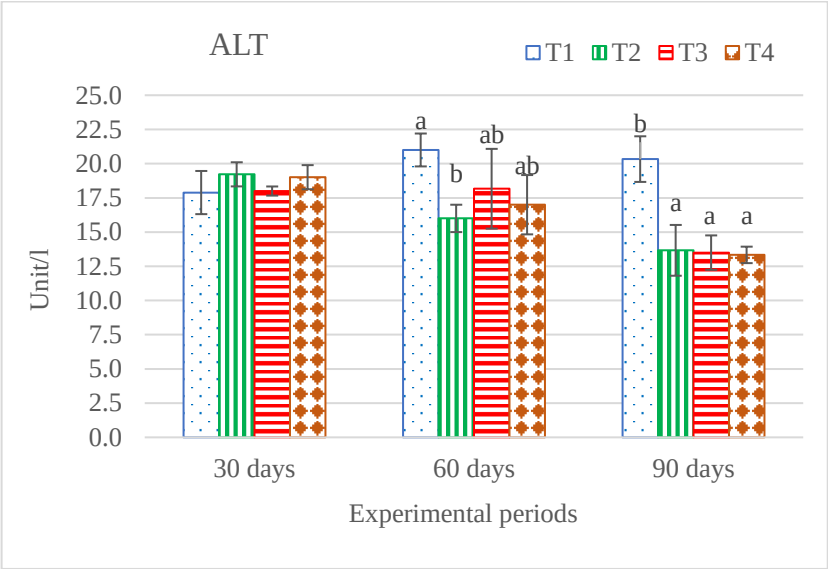
Figures 12 Total white blood cells, (WBC) which were fed different levels of *A. paniculata* extract for 30, 60, and 90 days: Significant differences ($p < 0.05$) is indicated by different letters.



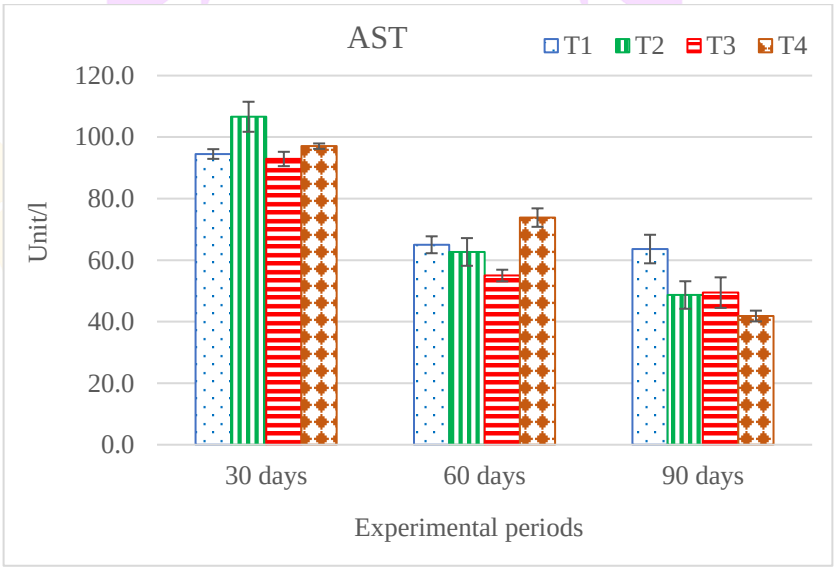
Figures 13 Hematocrit, (Hct) which were fed different levels of *A. paniculata* extract for 30, 60 and 90 days Significant differences ($p < 0.05$) is indicated by different letters.

4.4 Serum biochemical indices

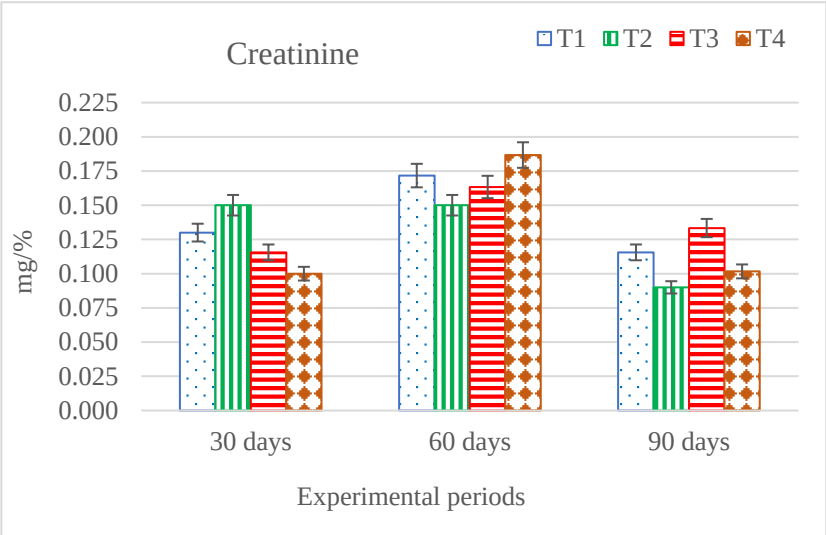
Biochemical indices, such as alanine transaminase (ALT), aspartate transaminase (AST), creatinine, glucose, cholesterol, LDL, HDL, total protein, triglyceride, albumin, globulin and alkaline phosphatase (ALP). The results showed that all parameters tested showed significantly different ($p < 0.05$) except for AST, creatinine, total protein, albumin, globulin, ALP and LDL. The parameters were significantly different, ALT, glucose and HDL were clearly shown on days 60 and 90 of the experiment, but triglyceride was significantly different on days 30, 60, and 90, while cholesterol was only significantly different on day 90. The ALT shows its highest value in T1 and its lowest in T2 on day 60. However, at day 90, the highest ALT was found in T1 while, lowest ALT was found in all treatment groups (Fig 14). Serum glucose revealed that started significantly different on days 60–90, the highest glucose was observed in T3, but the lowest level was found in T1 and T2 on day 60, while on day 90, the highest glucose was exhibited in all treatment groups and the lowest level was found in control group (Fig 15). Surprisingly, serum triglyceride can be reduced by the extract, on days 30 and 60 this parameter showed similar results, T4 group showed significantly lowest level but the highest level was found in T1, but the lowest level was found in all treatment groups when compared to the T1 group (Fig 19). Serum cholesterol revealed significantly different on day 90 of the experiment, The lowest and highest levels were observed in T4 and T1 groups, respectively (Fig 2 0). Serum HDL has been similar trended to glucose, this parameter showed initially different on days 60–90, the highest level was found in T4 and the lowest HDL was found in T1 and T2 on day 60, but the highest HDL on day 90 was observed in T4 followed by; T3, T2 and T1, respectively (Fig 22).



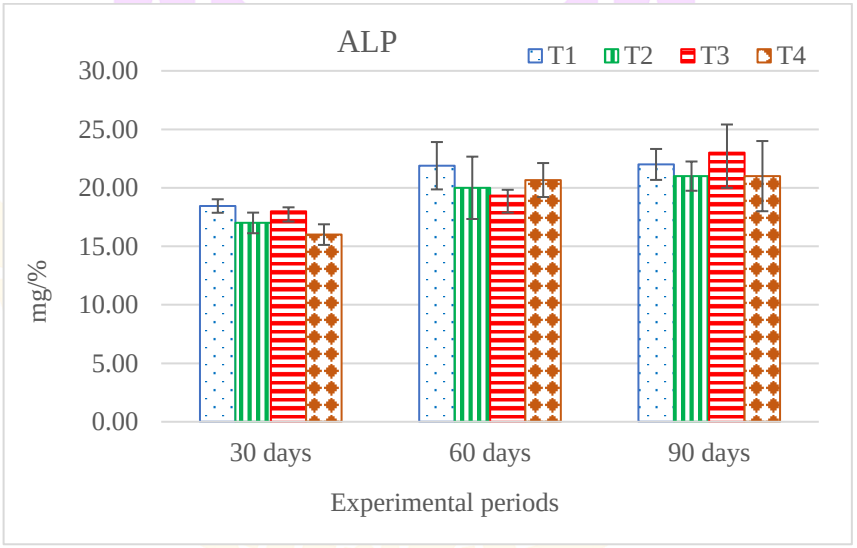
Figures 14 Serum alanine transaminase (ALT) in hybrid catfish, *Clarias macrocephalus X C. gariepinus.* Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



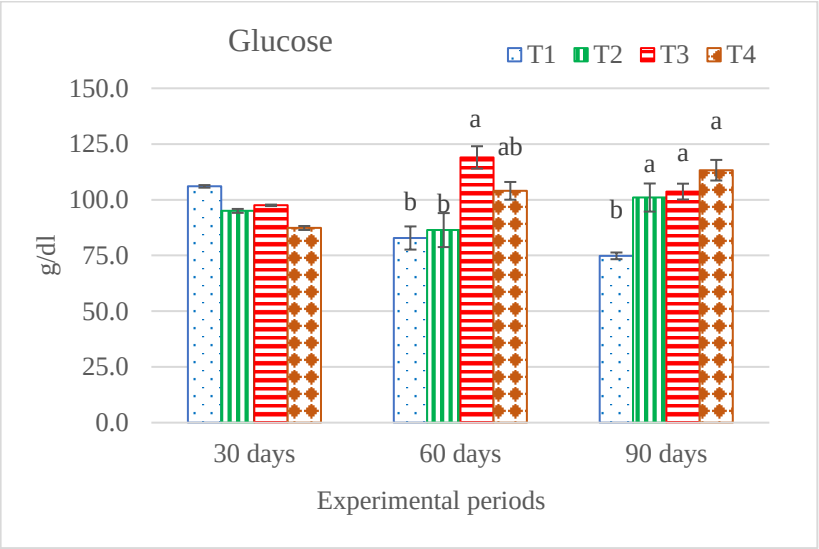
Figures 15 Serum aspartate transaminase (AST) in hybrid cafish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



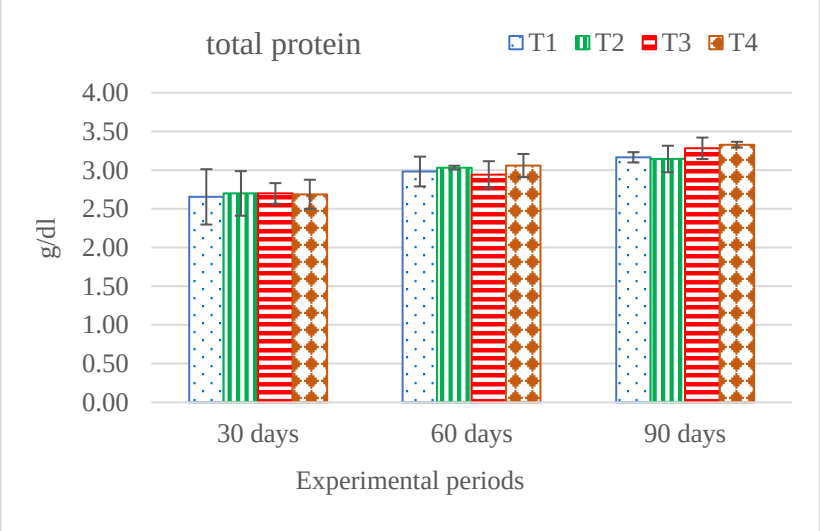
Figures 16 Serum creatinine in *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p > 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



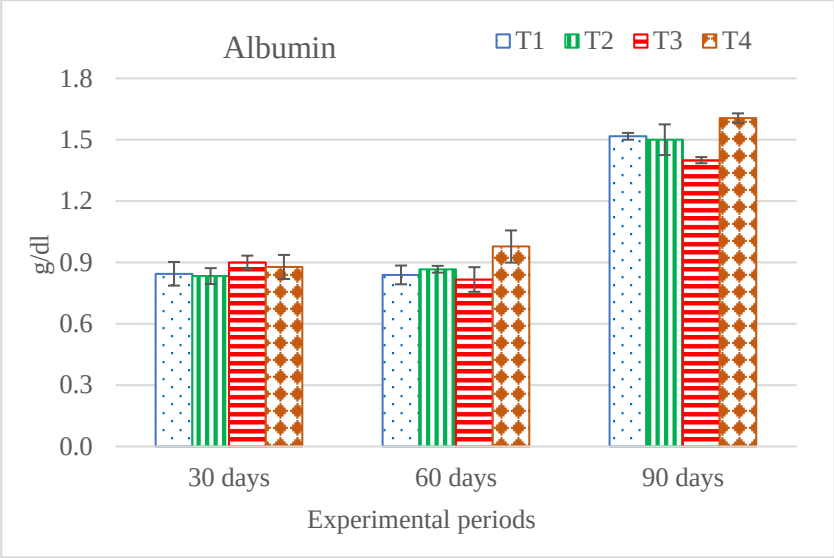
Figures 17 Serum alkaline phosphatase (ALP) in *Clarias macrocephalus X C. gariepinus.* Values are mean ± SD and different letters indicate difference ($p > 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



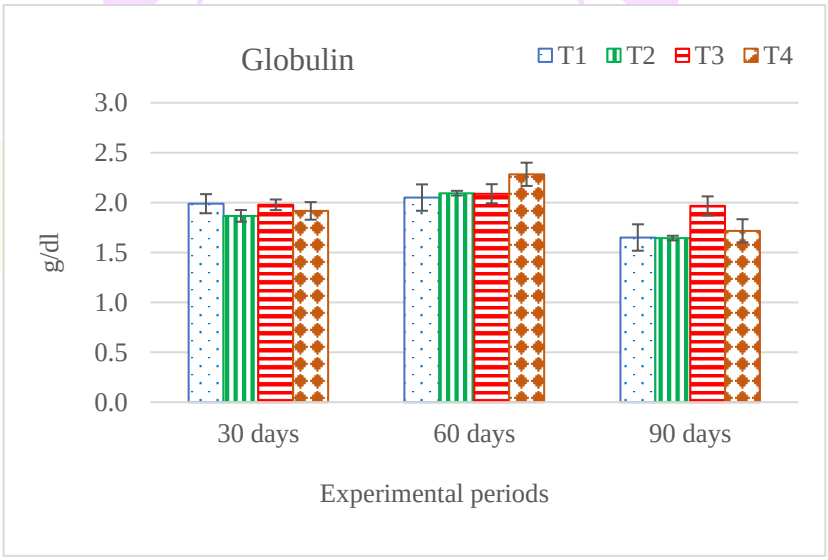
Figures 18 Serum glucose in *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



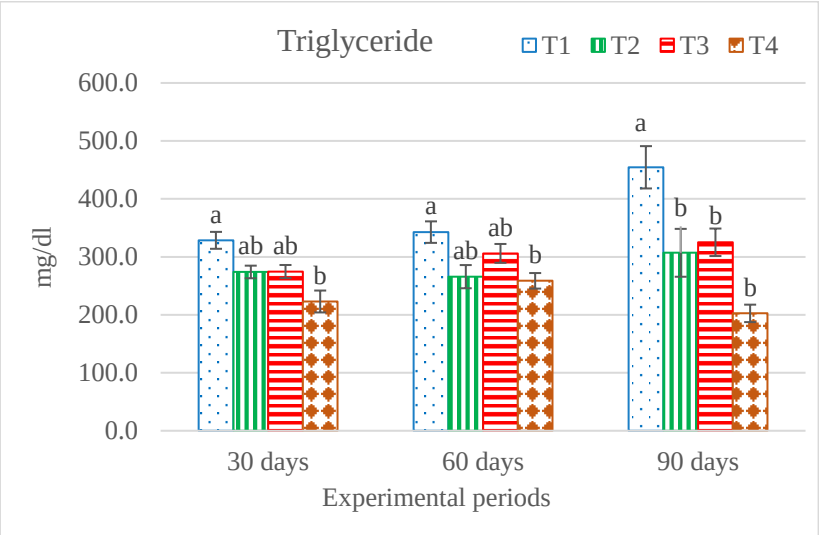
Figures 19 Serum total protein in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



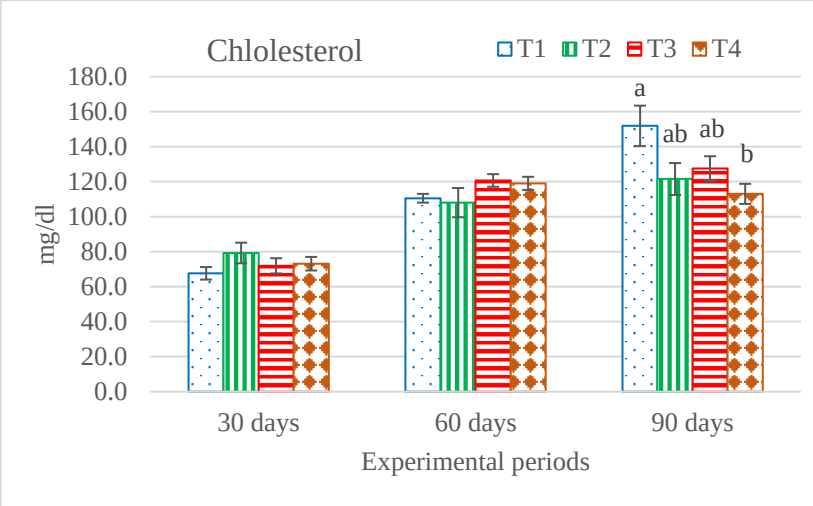
Figures 20 Serum albumin in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



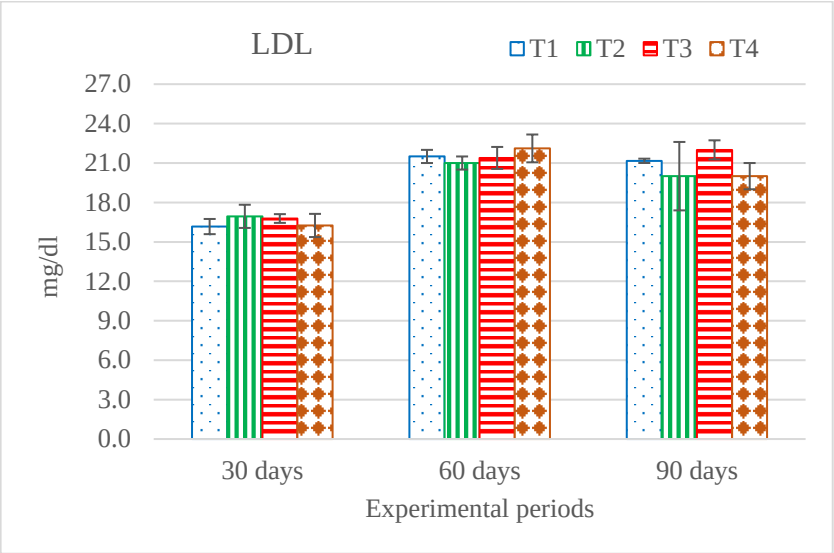
Figures 21 Serum globulin in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



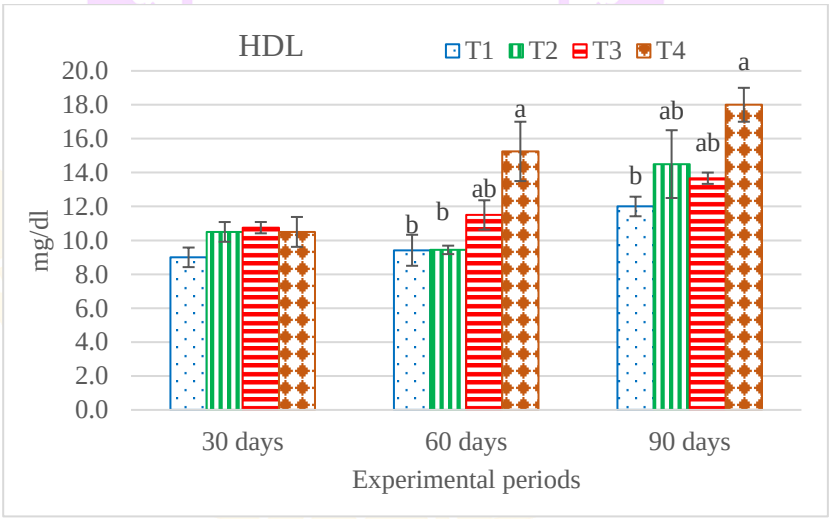
Figures 22 Serum triglycerides in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



Figures 23 Serum cholesterol in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



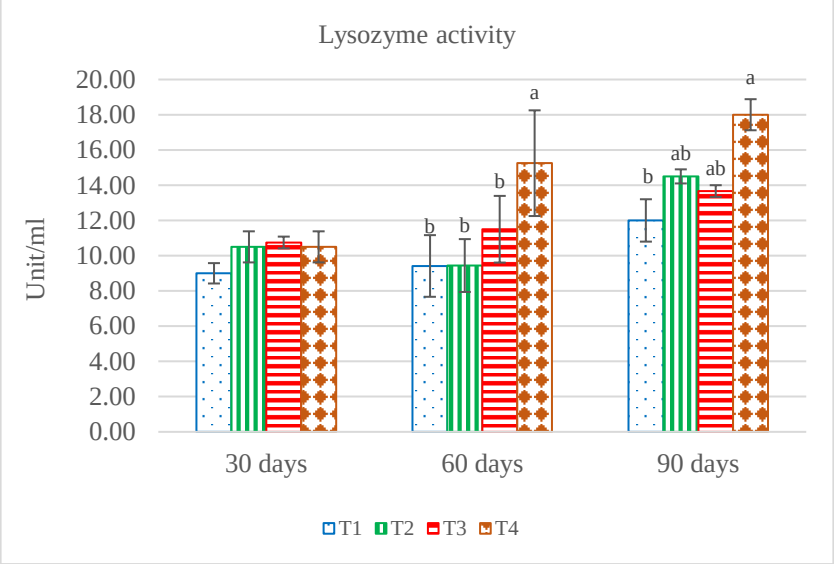
Figures 24 Serum low-density Lipoprotein (LDL) in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).



Figures 25 Serum high-density Lipoprotein (HDL) in hybrid catfish, *Clarias macrocephalus X C. gariepinus.* Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) andT4 (0.6 g/kg).

4.5 lysozyme activity

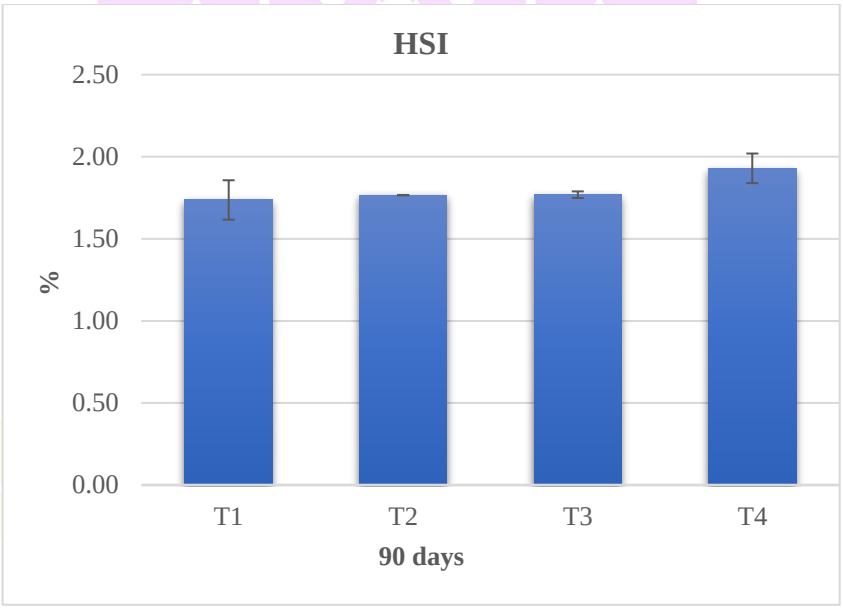
Serum lysozyme activity in all treatment groups (T2, T3 and T4) was significantly different ($P < 0.05$) and showed higher than in the control group (T1), as observed in all sampling days (Fig 2 6). On days 60 and 90, serum lysozyme activity in T4 group was consistently higher and significantly ($P < 0.05$) different from the values in T1 and T2 groups.



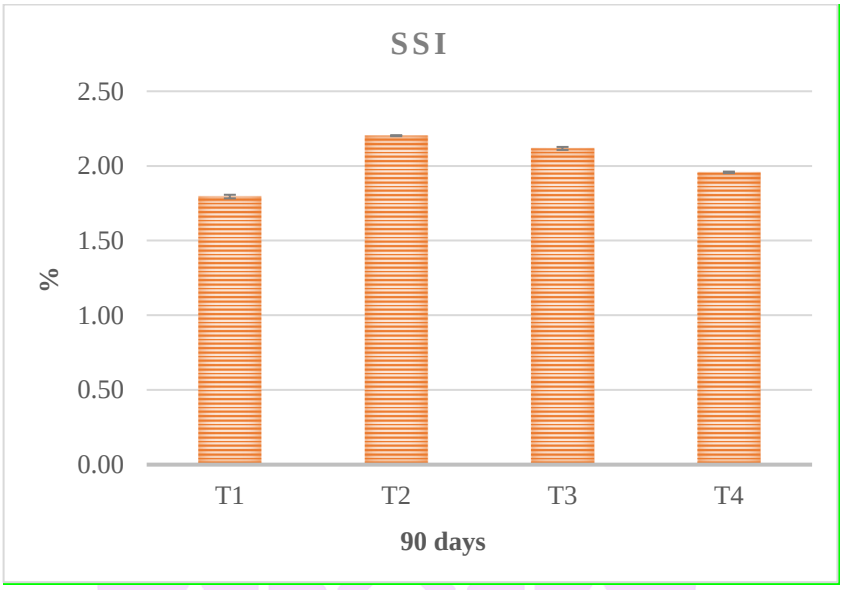
Figures 26 Serum lysozyme activity in hybrid catfish, *Clarias macrocephalus X C. gariepinus*. Values are mean ± SD and different letters indicate difference ($p < 0.05$). T1 (0.00 g/kg), T2 (0.2 g k), T3 (0.4 g /kg) and T4 (0.6 g/kg).

4.6 Organosomatic indices

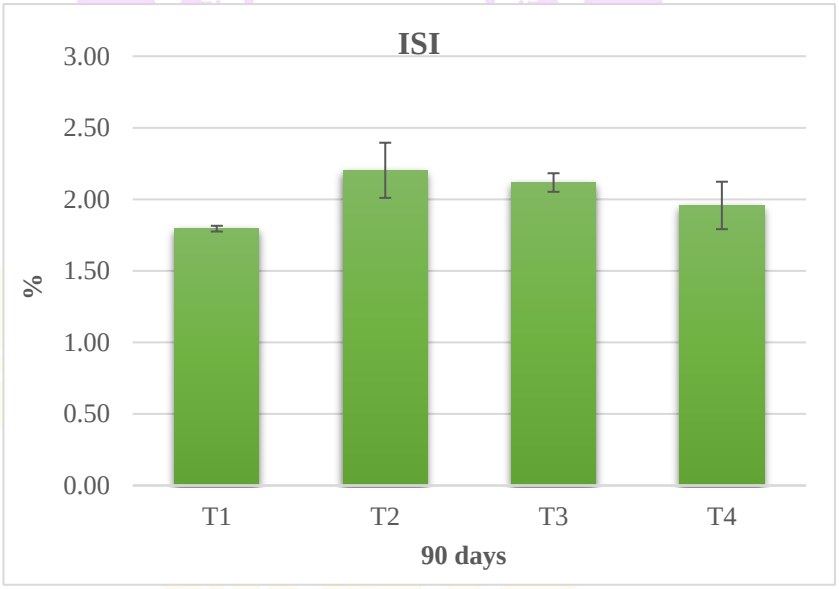
Organosomatic indices such as liver, spleen, kidney and intestine after the end of the experiment (90 days) in all groups were not significantly different ($P > 0.05$). While the trends of their indices showed that T4 group had the highest liver index (2.44%), while the control group had the lowest (2.12%). The spleen index were the highest and lowest levels in the groups T4 (0.11%) and T1 (0.096%), respectively. In addition, kidney index was found to be highest in group T4 (0.69%) but lowest in group T1 (0.59%). Similarly, the intestine index T4 group had the highest value at 2.74%, followed by groups T2, T1, and T3 as 2.55%, 2.52% and 2.22%, respectively (Fig 24–27).



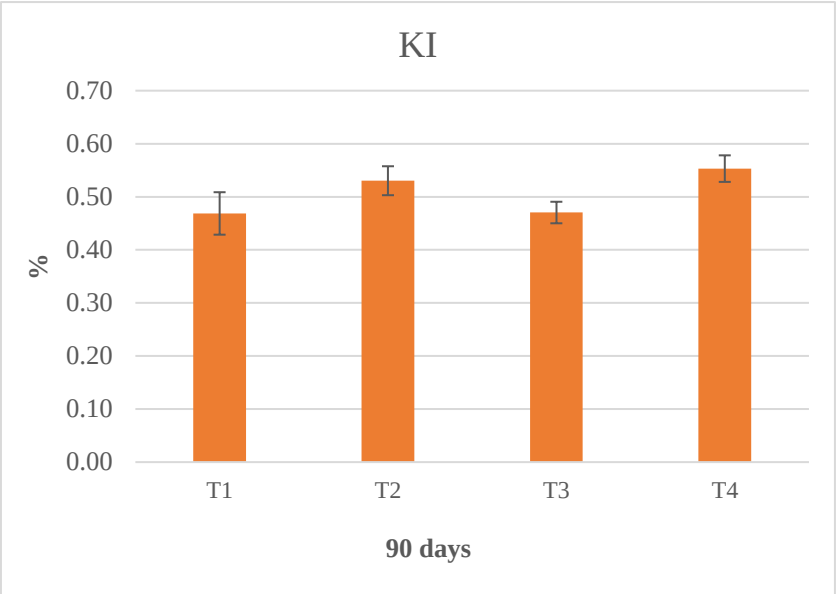
Figures 27 Hepatosomatic index (HSI), of hybrid catfish that were fed with different concentrations of *A. paniculata* extract for 90 days.



Figures 28 Spleen somatic index (SSI) of hybrid catfish that were fed with different concentrations of *A. paniculata* extract for 90 days.



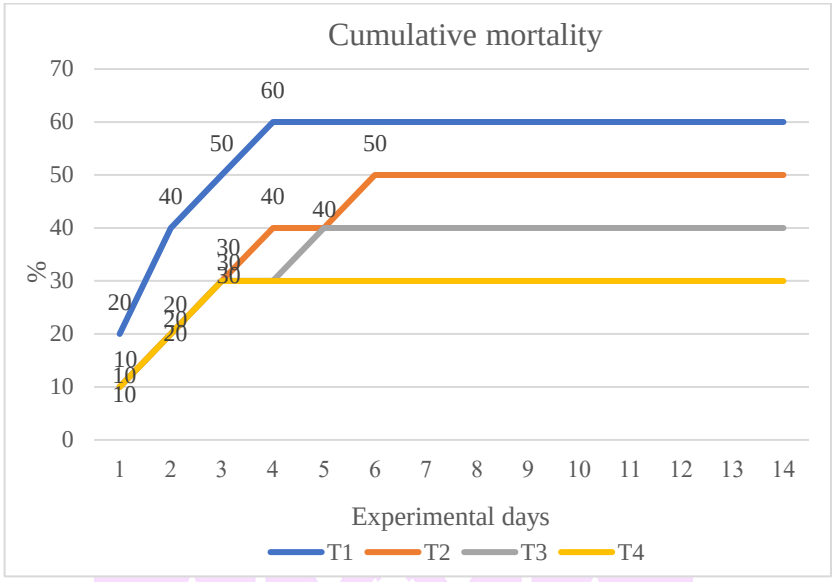
Figures 29 Intestinal somatic index (ISI) of hybrid catfish that were fed with different concentrations of *A. paniculata* extract for 90 days.



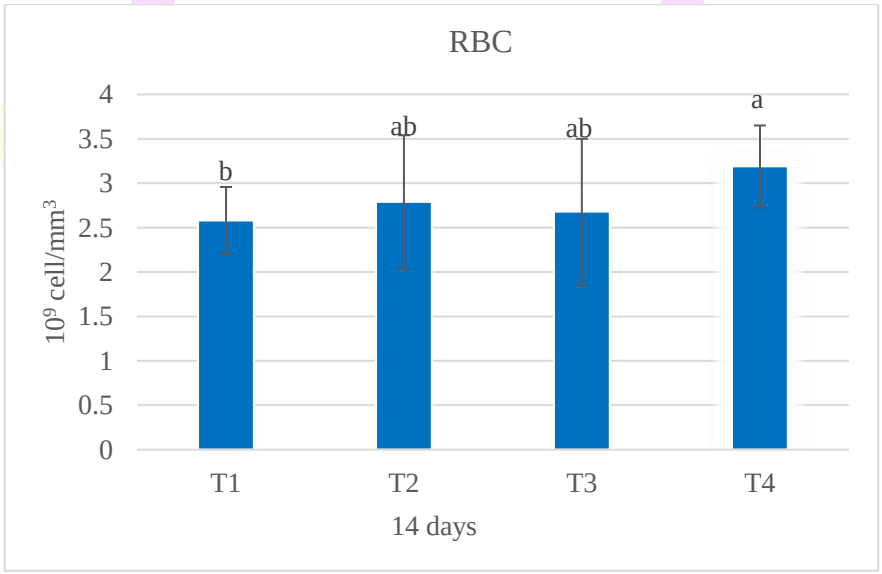
Figures 30 Kidney index (KI) of hybrid catfish that were fed with different concentrations of *A. paniculata* extract for 90 days.

4.7 Pathogenic bacterial challenge test

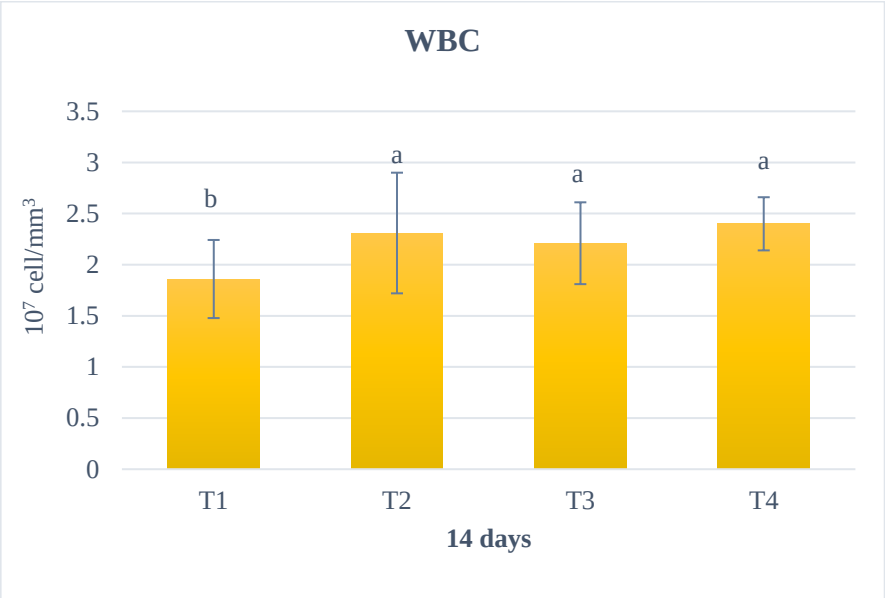
The observed cumulative mortality after *A. hydrophila* injections at 10^9 CFU/ mL for 14 days was the highest in T1 group at about 60 % when compared with the other group, and mortality was found on the first 4 days of the experimental period. During the pathogenic challenge study, we observed gasping, weak movement, rotting fins, and darkening of the skin. Cumulative mortality was recorded at 60%, 50%, and 30% in T3, T2 and T4, respectively (Fig 28). Hematological indices such as RBC, WBC and hematocrit percentage (Htc) were evaluated after challenge with *A. hydrophila* for 14 days, the results showed the same trend; all the treatment groups were significantly different ($P < 0.05$) from those in the control group (Fig 29–31). Lysozyme activity in fish survived after *A. hydrophila* injection at 10^9 CFU/ml. The lowest activity was found in T1 group when compared to the groups were received *A. paniculata* extract. (Fig 32).



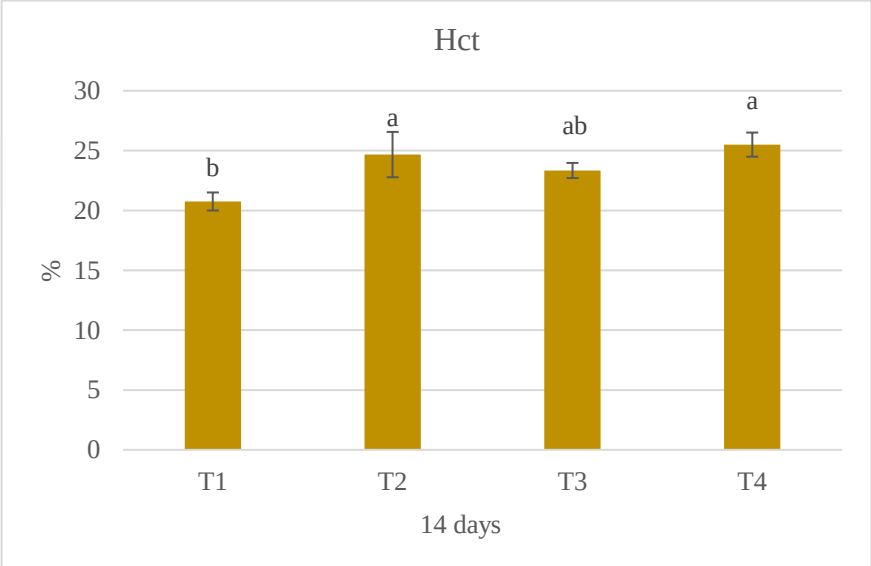
Figures 31 Cumulative mortality (%) of *Clarias macrocephalus X C. gariepinus* fed with the 4 different concentrations of *A. paniculata* extract against *A. hydrophila* for 14 days. T1 (0.00 g /kg), T2 (0.2 g/ kg), T3 (0.4 g/ kg) and T4 (0.6 g /kg).



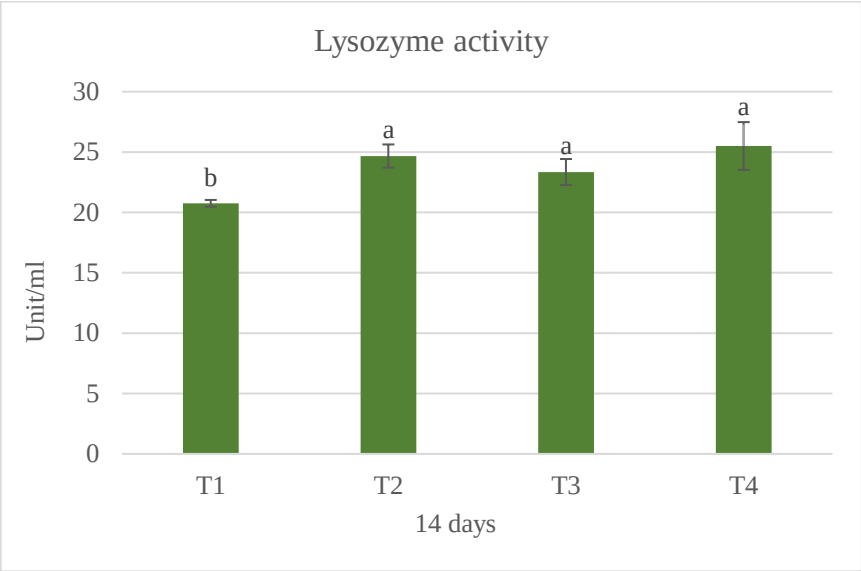
Figures 32 Total red blood cells, RBC of *Clarias macrocephalus X C. gariepinus* fed with the 4 different concentrations of *A. paniculata* after *A. hydrophila* injection at 10^9 CFU/mL for 14 days.



Figures 33 Total white blood cells of *Clarias macrocephalus X C. gariepinus* fed with the 4 different concentrations of *A. paniculata* after *A. hydrophila* injection at 10^9 CFU/mL for 14 days.



Figures 34 Hematocrit, Hct of *Clarias macrocephalus X C. gariepinus* fed with the 4 different concentrations of *A. paniculata* after *A. hydrophila* injection at 10^9 CFU/mL for 14 days.



Figures 35 Lysozyme activity of *Clarias macrocephalus X C. gariepinus* fed with the 4 different concentrations of *A. paniculata* after *A. hydrophila* injection at 10^9 CFU/mL for 14 days.

CHAPTER 5

DISCUSSION

5.1 Growth performance and survival rate

The using of *A. paniculata* extract in the diet of fish used in this experiment appeared to be significantly effective in terms of their growth performance, hematology, serum biochemical indices and lysozyme activity, in both the pre-challenge and post-challenge, *A. hydrophilla* injection, period. Our findings showed that a 95% ethanolic solution of *A. paniculata* extract significantly improved growth performance in the T4 group (0.6 g/kg). The results obtained from this phytochemical screening test confirmed the presence of diterpenes which is possibly including andrographolide. In addition, This may have been due to the existence of bioactive compounds in *A. paniculata*, such as andrographolide, deoxyandrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographide, isoandrographolide, coumarins, tannins, phenolics, terpenoids, lactones, steroids and sugars etc., that have been found in this plant species (Rajalakshmi, 2016; Nagajothi et al. 2018; Naomi et al., 2022). According to Maiti et al. (2021) reported that *Pangasianodon hypopthalmus* could improve weight gain, specific growth rate and feed conversion ratio after fed 2% *A. paniculata* leaf extract. Similarly, Basha et al. (2013) revealed that *Labeo rohita* fed an andrographolide diet improved WG, SGR, protein efficiency ratio and decreased feed conversion ratio. In addition, *Pagrus major* fed with medicinal herbs increased WG, FE and survival rates above those found in the control group (Ji et al., 2007). Moreover, Mishra et al. (2023) reported that andrographolide can stimulate growth performance in *Cyprinus carpio* due to an increase in its ability to use of protein, energy, digestion and better absorption of nutrients as a result of its inclusion in their diets. In addition, The *A. paniculata* can reduce the growth rate of various pathogenic bacteria in *Penaeus monodon*, increasing their survival chances due to this herb's effectiveness in improving a fish's ability to digest and absorb nutrients and stimulate humoral immunity by enhancing the microbial killing activity of red blood cells and cell

phagocytosis (Citarasu et al., 2003). Meanwhile, Immanuel et al., (2009) reported that Nile tilapia fed *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera* and *Zingiber officinale* can stimulate WG, SGR, FE and reduce FCR. Besides, Shi et al. (2020) reported that an increase in WG, SGR, ADG and reduced FCR may be due to increased levels of andrographolide in the diet, which can lead to better absorption of nutrients in the intestines of Monopterus albus. According to Abdel Tawwab et al. (2010), the use of green tea, Camellia sinensis at a level 0.5g/kg of feed can enhance growth performance due to the palatability or attractiveness of the diet, which in turn causes increased feed intake and enhanced growth performance in Nile tilapia. Moreover, various other plant extracts have been shown to improve growth performance in fish, such as an *Euphorbia hirta* plant leaf extract supplemental diet of 300 mg/kg, which has a positive effect on hybrid catfish, improving their growth performance, survival rate and feed utilization (Panase et al., 2018a). This may be because bioactive substances can improve growth performance by promoting the synthesis of digestive enzymes, bile, musus and as such can be effective as feed additives in that they increase feed intake and ingestion (Rudy et al., 2018; Wang et al., 2018; Xu et al., 2020; Abdel-Latif et al., 2022). According to Baba et al. (2016), *Avena sativa* extract can be used to improve WG and SGR and decrease FCR in *Cyprinus carpio* because the metabolites of plants can stimulate growth and immunity via innate adaptive immune responses and can trigger immune cell activity, enhance phagocytosis and enhance the secretion of inflammatory markers to resist various pathogens in fish. Although we do not investigate the effect of *A. paniculata* on digestive enzyme, we found that *Zingiber officinalis* and *Cyanodon dactylon* supplementations could be increased SGR and decreased FCR in *Macrobrachium rosenbergii*, because of an increase in digestive enzyme secretion that can result in improvements in digestibility, stimulating the appetite and increasing food consumption and efficiencies (El-desouky et al. 2021). Furthermore, Ghosal et al. (2020) revealed that *Basella alba*, *Tribulus terrestris*, *Mucuna pruriens* and *Asparagus racemosus* can stimulate growth performance in Nile tilapia as it helps the fish to control reproduction and enhances growth and innate immunity because, all four plant extracts contained phytoconstituents such as saponins, alkaloids and tannins that have been reported to act as feed intake deterrents and

digestive enzyme inhibitors in Nile tilapia. Importantly though, Peng et al. (2021) revealed that condensed tannins did not impact on growth performance, but rather enhanced animal health by improving the intestinal microbial ecosystem in *Lateolabrax japonicus*. Meanwhile, *Astragalus membranaceus* extract can be used to improve growth performance in *Pangasianodon hypophthalmus* due to the presence of phenolic acids and flavonoids that can increase digestive enzyme activities, voluntary feeding intake, feed efficiency, and improve protein retention (Abdel-Latif et al., 2022). Besides, Ahmadifar et al. (2021) reported that polyphenols have affected the growth of gene expression in Huso huso, and this may be because tannins help in activating growth hormones (GH) and insulin-like growth factor-I (IGF-I) genes, which are known to be important genes for growth.

5.2 Hematological indices

Hematological indicators often alter in response to stress, illness, and supplemental diet circumstances; however, RBC, WBC and Htc are mostly impacted by dietary treatments (Reverter et al., 2014; Hassaan et al., 2019). In addition, erythrocytes and hemoglobin are essential in the transfer of oxygen and carbon dioxide (Nya et al., 2009). The results of our study showed that all groups receiving *A. paniculata* extract had higher RBC, WBC and Hct than the control group, but the group receiving *A. paniculata* extract, T4, had the highest values. According to Maiti et al. (2021) who reported that *A. paniculata* leaf extract could increase RBC in *Pangasianodon hypopthalmus* due to its iron content, iron being a substance that can produce red blood cells. According to Prasad and Priyanka (2011), *A. paniculata* contains trace minerals and iron. Besides, Velichkova et al. (2019) reported that increases in RBC in *Cyprinus carpio* may be because of mineral and iron constituents that can stimulate the excitability of muscles and blood coagulation. However, *Aloe vera* extract can also increase the RBC and Htc of *C. carpio* after an *A. hydrophila* infection. In addition, polysaccharides and iron in herbal extracts have also been associated with increased erythropoiesis and increased Htc levels. (Alishahi et al., 2010). According to, Binaii et al. (2014) reported that *Urtica dioica* extract can improve RBC and Hct in *Huso huso* due to the presence of vitamins and minerals from the extract that aide by increasing hematopoiesis. Furthermore, *C. carpio* fed with *Aegle marmelos* extract, hematology was

increased due to iron, which induces erythropoiesis and lymphopoiesis, resulting in an increase in RBC, Hct and WBC respectively (Prasad et al., 2011).

5.3 Serum biochemical indices

Biochemical indices indicators are commonly used to assess the health of animals, including fish. Our results showed that by day 60, ALT values began to differ, but a significant difference was clearly seen on day 90, with the group receiving the *A. paniculata* extract, group T4, which had the lowest values compared to the control group. This result is very similar to the findings of Tan et al., (2017) who reported that dandelion extracts could help Trachinotus ovatus decrease ALT levels, and that this may have been because bioactive compounds, phenolics anti–radicals and antioxidants, prevent lipid peroxidation of cell membranes and inhibit the release of ALT enzymes into the plasma. Therefore, this was found to be beneficial to liver health and had a hepatoprotective effect. Since glucose molecules play a significant role in animal bioenergetics, glucose is generally recognized to be a critical product of the cellular respiration process, which is transferred to ATP synthesis (Lucas, 1996). In this study, all the extract–treated groups had different serum glucose levels at day 60 and the difference was clearly seen at day 90, with group T4 showing the highest values. Glucose levels are indicators of stress levels, higher glucose levels are generally maintained in fish due to glycogen breakdown in the liver, after which the glucose molecules are converted into energy via glycogenolysis as part of the cellular respiration process (Vijayan et al., 1997). According to Lin et al. (2016), polyphenolic compounds can be improved the catalytic activity of glucose phosphorylation; what is more, natural plant extracts, for example eugenol, can affect glucose metabolization. In this study, cholesterol and triglyceride levels were decreased and showed their lower levels in group T4. Cholesterol levels in serum can be altered by diet, enzyme activities and hepatic activities. Moreover, cholesterol levels can be changed by the sexual cycle of fish because cholesterol is the precursor of the five major classes of steroid hormones (Berg et al., 2002). Meanwhile, the difference in cholesterol of serum may be the existence of plant sterols such as stigmasterol, campesterol and phenolic compounds (Frémont et al., 2000). According to Binaii et al. (2014), *Urtica dioica* extract can decrease serum cholesterol and triglyceride

levels in *Huso huso* due to the presence of stigmasterol, campesterol and phenol compounds. Beyond this, Chickens fed a Carvacrol Lowers diet can show a decrease in serum triglycerides, due to the steroids and phenolics that may affect lipid synthesis rather than cholesterol synthesis (Lee et al., 2003). The levels of HDL in serum are important parameters of lipid metabolism in animals and the lipid-lowering effect in serum is beneficial to health Adler and Holub (1997). Our study indicated that the LDL levels in all experimental groups were not significantly different because the extract had no effect on this serum parameter. On the other hand, the HDL level of all groups fed with *A. paniculata* extract was affected, especially on day 90 where it was shown to be significantly different when compared the control group, and an even higher level was observed in the T4 group. This is in concordance with the findings of Brown and Goldstein (1984) who reported that *P. hypophthalmus* fed with *Garcinia gummi-gutta* extract showed an increase in HDL values. Besides this, Pourmoghim et al. (2015) reported that *Oncorhynchus mykiss* fed with *Origanum vulgare* extract can show improved HDL values. This is because bioactive compounds, such as minerals and iron, stimulate HDL activity by transporting cholesterol from the blood to the liver.

5.4 Lysozyme activity

Lysozyme activity serves as a primary defense, providing humoral-specific immunity to set up cellular defense mechanisms. In addition, Lysozyme is found in the serum and mucus of fish (Ellis, 1999). This study had indicated that all the extract-treated groups had different lysozyme activity levels at day 60 and that the difference was very clearly seen on day 90; group T4 had the highest values compared to other groups. Agreement with the findings of Maiti et al. (2021) reported that *P. hypopthalmus* fed with *A. paniculata* leaf extract could increase serum lysozyme activity due to the bioactive compounds of this herb, which could stimulate the non-specific immune system response naturally occurring in fish. In addition, it improves cellular defense mechanisms and has the ability to destroy the cell walls of some pathogens. According to Basha et al. (2013), feeding the andrographolide in *Labeo rohita* can increase lysozyme activity due to the andrographolide stimulating an increase in the mechanism of a non-specific immune

response. In addition, it breaks down the cell walls of both gram–negative and positive gram–positive bacteria.

5.5 Organosomatic indices

Organosomatic indices such as hepatosomatic index (HSI), spleen somatic index (SSI), kidney index (KI) and intestinal somatic index (ISI) were used to evaluate the results of this study. This is because the indices have effects, though changes in organosomatic size and are influenced by various factors such as water quality, stocking density, feed or supplemental diets and stress (Akani and Daka 2015; Kareem et al., 2016). Our study indicated that the organosomatic indices in all experimental groups were not significantly different ($P > 0.05$). This is in agreement with the findings of Gabriel et al. (2015), who reported that Oreochromis niloticus fed an Aloe vera supplement showed VI, FI and KI were not significantly different. Meanwhile, (Bahabadi et al., 2014) reported that feeding *Oncorhynchus mykiss* with *Achillea millefolium* extract had no effect on HSI, ISI and SSI. The reason the organs have value is not different because of the bioactive compound but because of this herb's lack of negative effect and safety on organosomatic indices in fish.

5.6 Pathogenic challenge test

Our study showed that all groups that were fed with *A. paniculata* extract suppleme showed a lower cumulative mortality rate when compared with the control group. After the 90–day feeding trial, experimental fish from each group were injected with an *A. hydrophila* suspension at 10^9 CFU/mL for 14 days. Mortality in all these groups was found only in the first 4 days of the experiment and the highest cumulative mortality rate was observed in the control group, while the lowest cumulative mortality rate was found in group T4. This is in agreement with the findings of Maiti et al. (2021), where it was reported that *P. hypopthalmus* fed with *A. paniculata* extract showed a reduction in cumulative mortality rate when infected with *A. hydrophila*. In addition, Basha et al. (2013) have demonstrated that andrographolide can resist *A. hydrophila* in *Labeo rohita* because andrographolide stimulates non–specific immune response mechanisms. Thus, all the extracted groups had better body readiness than the control group for resistance to *A. hydrophila*.

Our study indicates that all groups that were fed with *A. paniculata* extract after injection with *A. hydrophila* for 14 days had higher values of hematological indices when compared to the control group. According to Harikrishnan et al. (2010) it was revealed that bacterial infection has a direct effect on an organism's hematology and serum parameters because of enzymatic digestion of the erythrocytes, which leads to reduced WBC and Hct levels. In addition, Palanikani et al. (2018) reported the effect of the herbal supplement *A. paniculata* for control of *A. hydrophila* and *A. veronii* in *Catla–catla*. Similarly, Palanikani et al. (2020) showed that *A. paniculata* extract can control *A. hydrophila* in *Labeo rohita*, which may have minerals and iron that can produce red blood cells and increase oxygen transportation throughout the body of the fish, resulting in an increase in RBC and increased levels of WBC as a result of increased phagocytic activity.

All groups that were fed *A. paniculata* extract after injection with *A. hydrophila* for 14 days had higher lysozyme activity values than the control group. Similar results were found by Maiti et al. (2021) who reported that supplementation with *A. paniculata* leaf extract could improve lysozyme activity on *P. hypopthalmus* after being infected with *A. hydrophila*. Moreover, Basha et al. (2013) reported that supplementation with andrographolide was able to increase the activity of lysozyme on *Labeo rohita* after infection with *A. hydrophila*. This was because, prior to infection, all the groups of fish that received the extract had non–specific immune system stimulation, and when infected, all groups receiving extracts had better body readiness than the control groups. In addition, Palanikani et al. 2020 reported that the ability of *A. paniculata* extract to increase the phagocytic index (monocyte, neutrophil and lymphocyte) and act as immune cells by engulfing and destroying the pathogen; these are vital components of the non–specific immune system.

5.7 Conclusion

The results obtained from our study demonstrated that a 95% ethanolic extract solution of *A. paniculata* for three months can improve the growth performance of hybrid catfish, *C. macrocephalus x C. gariepinus* due to the fact that andrographolide can stimulate feed utilization, efficiency of protein utilization, digestion and better absorption of nutrients. In addition, *A.paniculata* in feed can stimulate hematological indices because mineral and iron

constituents can stimulate the excitability of muscles and blood coagulation. Meanwhile, this herb also has a positive effect on biochemical indices because of bioactive compounds such as those minerals and iron found in this present study. Besides, *A.paniculata* extract can increase the proper functioning of the immune system, as well as improve innate immune responses and disease resistance in *A. hydrophila*. Moreover, *A. paniculata* extract can be introduced into feed without impacting liver, kidney, spleen and intestine indices. The appropriate concentration to use is suggested by our research as 0.6 g/kg, because it is safe and no adverse effects were discerned that would prevent the production of healthy fish for human consumption

BIBLIOGRAPHY

Abdalla, A. A., Mustafa, M. I., & Makhawi, A. M. J. b. (2020). **Phytochemical screening and antimicrobial activities studies of Acacia nilotica fruit cover.** 2020.2002. 2011.943456.

Abdel–Latif, H. M., Ahmed, H. A., Shukry, M., Chaklader, M. R., Saleh, R. M., & Khallaf, M. A. (2022). *Astragalus membranaceus* extract (AME) enhances growth, digestive enzymes, antioxidant capacity, and immunity of *Pangasianodon hypophthalmus* juveniles. **Fishes,** 7(6), 319.

Abdel–Tawwab, M., Ahmad, M. H., Seden, M. E., & Sakr, S. F. (2010). Use of green tea, *Camellia sinensis* in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection. **Journal of the World Aquaculture Society,** 41:203–13.

Adan, R. I. Y. (2000). Catfish culture in Southeast Asia. **SEAFDEC Asian Aquaculture,** 22(1), 16–17.

Adler, A. J. & Holub. B. J. (1997). Effect of garlic and fish–oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemic men. **Am J Clin Nutr.** 65(2):445–50.

Ahmadifar, E., Fallah, H. P., Yousefi, M., Dawood, M. A. O., Hoseinifar, S. H., Adineh. H., & Yilmaz, S. (2021). The Gene Regulatory Roles of Herbal Extracts on the Growth, Immune System, and Reproduction of Fish. **Animals,** 11, 2167.

Akani, N.P., & Daka, E.R. (2015). Evaluation of weight changes, condition factor and organosomatic indices of *Clarias gariepinus* exposed to sub–lethal concentrations of an oilfield wastewater. **Curr Stud Comp Educ Sci Technol,** 2:338–354.

Alemu, Y. (2006). **Hematology.**

Al–Khshali, M. S., & Al Hilali, H. A. (2019). Some physiological changes (ALP, AST and ALT) of common carp (Cyprinus carpio) caused by high salinity. **Biochemical & Cellular Archives,** 19(2).

Alishahi, M., Ranjbar, M.M., Ghorbanpour, M., Peyghan, R., Mesbah, M., & Razi, J.M.

(2010). Effects of dietary *Aloe vera* on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). **Int J Vet Res,** 4:189–195.

Ashton, N. (2007). Physiology of red and white blood cells. **Anaesthesia & Intensive Care Medicine,** 8(5), 203–208.

Bagenal, T. (1978). **Methods for assessment of fish production in fresh waters–3.**

Bahabadi, M.N., Banaee, M., Taghiyan, M.,&Haghi, B.N. (2014). Effects ofdietary administration of yarrow extract on growth performance and blood biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). **Int J Aquat Biol,** 2:275–285.

Basha, K. A., Raman, R. P., Prasad, K. P., Kumar, K., Nilavan, E., & Kumar, S. (2013). Effect of dietary supplemented andrographolide on growth, non–specific immune parameters and resistance against *Aeromonas hydrophila* in *Labeo rohita* (Hamilton). **Fish & Shellfish Immunology,** 35 (5):1433–41.

Bayne, C.J. (2003). Co–evolution of innate and adaptive immunity. **Integr Comp Biol** 2003; 43: 291–299.

Belinskaia, D. A., Voronina, P. A., Shmurak, V. I., Jenkins, R. O., & Goncharov, N. V. (2021). Serum albumin in health and disease: esterase, antioxidant, transporting and signaling properties. **International journal of molecular sciences,** 22(19), 10318.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). **Biochemistry,** W. H. New York: Freeman and Company: New York.

Bhaskar–Reddy, M. V., Kishore, P. H., Rao, C. V., Gunasekar, D., Caux, C., & Bodo, B. (2003). New 2 '–Oxygenated Flavonoids from *Andrographis affinis*. **Journal of natural products,** 66(2), 295–297.

Binaii, M., Ghiasi, M., Farabi, S. M. V., Pourgholam, R., Fazli, H., Safari, R., & Bankehsaz, Z. (2014). Biochemical and hemato–immunological parameters in juvenile beluga (Huso huso) following the diet supplemented with nettle (Urtica dioica). **Fish & shellfish immunology,** 36(1), 46–51.

Bower, C.K., Avena–Bustillos R.J., Olsen, C.W., McHugh T.H., & Bechtel P.J. (2006) Characterization of ¢sh–skin gelatin gels and ¢lms containing the antimicrobial enzyme lysozyme. **Journal of Food Science 71,** M141^M145.

Brown, M. S., & Goldstein, J. L. (1984). How LDL Receptors Influence Cholesterol and Atherosclerosis. **Scientific American,** 5:58–69.

Bulfon, C., Volpatti, D., & Galeotti, M. (2015). Current research on the use of plant–derived products in farmed fish, **Aquacult. Res.** 46: 513–551. https://doi.org/10.1111/are.12238

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., ElSohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. **Evidence–based complementary and alternative medicine,** 2014.

Chaivichoo, P., Koonawootrittriron, S., Chatchaiphan, S., Srimai, W., & Na–Nakorn, U. (2020). Genetic components of growth traits of the hybrid between ♂ north African catfish (*Clarias gariepinus* Burchell, 1822) and ♀ bighead catfish (*C. macrocephalus* Günther, 1864). **Aquaculture 521,** 735082. https://doi.org/10.1016/j. aquaculture.2020.735082.

Chakraborty, S.B., Horn, P., & Hancz, C. (2014). Application of phytochemicals as growth promoters and endocrine modulators in fish culture, Rev. **Aquacult.** 6: 1–19. https://doi.org/10.1111/raq.12021

Chang, K.T., Lii, C.K., Tsai, C.W., Yang, A.J., & Chen, HW. (2008). Modulation of the expression of the π class of glutathione S–transferase by *Andrographis paniculata* extracts and andrographolide. **Food Chem Toxicol,** 46:1079–1088.

Chao, W. W., & Lin, B. F. (2010). Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). **Chinese medicine,** 5, 1–15.

Charoendat, U., Chumchareon, M., & Phumee, P. (2016). Effects of Creat (*Andrographis paniculata* Wall. Ex Nees) Extract on Growth Performance and Bacterial Disease Resistance in Pacific White Shrimp (*Litopenaeus vannamei Boone*). **RMUTSV Research Journal,** 8(2), 190–202.

Cheung, H. Y., Cheung, C. S., & Kong, C. K. (2001). Determination of bioactive diterpenoids from *Andrographis paniculata* by micellar electrokinetic chromatography. **Journal of**

Chromatography A, 930(1–2), 171–176.

Citarasu, T., Venkatramalingam, K., Micheal–Babu, M., Jeya–Sekar, R. R., & Petermarin, M. (2003). Influence of the antibacterial herbs, *Solanum trilobatum, Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. **Aquaculture International,** 11: 583–595.

Dada, A. A., & Sonibare, O. F. (2015). Effect of dietary administration of the herbal additive siamweed (*Chromolaena odorata*) on growth performance and haematological change in *Clarias gariepinus* fingerlings. **Journal of Fisheries,** 3(1), 221–226.

Das, S., Neogy, S., Gautam, N., & Roy, S. (2009). In vitro nitotine induced superoxide mediated DNA fragmentation in lymphocytes: protective role of *Andrographis paniculata* Nees. **Toxicol in Vitro,** 23:90–98.

Densmore, C.L., & Green, D.E. (2007). **Diseases of amphibians.** ILAR J 48:235–254.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. **African journal of biotechnology,** 4(7), 685–688.

El–Desouky, H., El–Asely, A., Shaheen, A. A., & Abbass, A. (2012). Effects of Zingiber officinalis and Cyanodon dactylon on the growth performance and immune parameters of *Macrobrachium rosenbergii*. **World Journal of Fish and Marine Sciences,** 4(3), 301–307.

Ellis, A. E. (1999). Immunity to bacteria in fish. **Fish & shellfish immunology,** 9(4), 291–308.

Ellis, D. S., Simpson, I. H., Francis, D. P., Knobloch, J., Bowen, E. T., Lolik, P. A. C. I. F. I. C. O., & Deng, I. M. (1978). Ultrastructure of Ebola virus particles in human liver. **Journal of Clinical Pathology,** 31(3), 201–208.

Food and Agriculture Organization of the United Nations (FAO) (2020). **Fishery and aquaculture statistics.** Global aquaculture production 1950–2018 (FishstatJ).

Frémont, L., Gozzelino, M. T., & Linard, A. (2000). Response of Plasma Lipids to Dietary Cholesterol and Wine Polyphenols in Rats Fed Polyunsaturated Fat Diets. **Lipids,** 36 (9): 991–9.

Janeway Jr, C. A., & Medzhitov, R. (2002). Innate immune recognition. **Annual review of**

immunology, 20(1), 197–216.

Jayakumar, T., Hsieh, C.Y., Lee, J.J., & Sheu, J.R. 2013. Experimental and clinical pharmacology of *Andrographis paniculata* and its major bioactive phytoconstituent Andrographolide. **Evid base Compl Alternative Med,** 2013:846740. https://doi.org/10.1155/2013/846740

Klinken, S. P. (2002). Red blood cells. T**he international journal of biochemistry & cell biology,** 34(12), 1513–1518.

Kurosaki, T., Kometani, K., &Ise, W. (2015). Memory B cells. **Nature Reviews Immunology,** 15(3), 149–159.

Gabriel, N.N., Qiang, J., He, J., Ma, X.Y., Kpundeh, M.D., & Xu, P. (2015). Dietary *Aloe vera* supplementation on growth performance, some haemato–biochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). **Fish & Shellfish Immunology,** 15: 1–11.

Gabriel, N. N., Wilhelm, M. R., Habte–Tsion, H. M., Chimwamurombe, P., Omoregie, E., Iipinge, L. N., & Shimooshili, K. (2019). Effect of dietary *Aloe vera* polysaccharides supplementation on growth performance, feed utilization, hemato–biochemical parameters, and survival at low pH in African catfish (*Clarias gariepinus*) fingerlings. **International Aquatic Research,** 11, 57–72.Galindo

Gupta, S., Choudhry, M. A., Yadava, J. N. S., Srivastava, V., & Tandon, J. S. (1990). Antidiarrhoeal activity of diterpenes of *Andrographis paniculata* (Kal–Megh) against Escherichia coli enterotoxin in in vivo models. **International Journal of Crude Drug Research,** 28(4), 273–283**.**

Gupta, S., Mishra, K.P., & Ganju, L. (2017). **Broad–spectrum antiviral properties of andrographolide.** Arch Virol 162(3):611–623. https://doi. org/10.1007/s00705–016–3166–3

Ghosal, I., Mukherjee, D., & Chakraborty, S. B. (2021). The effects of four plant extracts on growth, sex reversal, immunological and haemato**-**biochemical parameters in Nile tilapia, *Oreochmomis niloticus* (Linnaeus, 1758). **Aquaculture Research,** 52(2), 559–576.

Hadidi, S., Gavin, W.G., Timothy, J.W., Jeffrey, T.S., & Gregory, D.W. (2008). Spleen size predicts resistance of rainbow trout to Flavobacterium psychrophilum challenge. **J Immunol,** 180:4156–4165.

Harikrishnan, R., Balasundaram, C., & Heo, M.S. (2010). Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila* . **Fish Shellfish Immunol,** 28:354–361.

Harikrishnan, R., Kim, J. S., Kim, M. C., Balasundaram, C., & Heo, M. S. (2011). Lactuca indica extract as feed additive enhances immunological parameters and disease resistance in *Epinephelus bruneus* to *Streptococcus iniae*. **Aquaculture,** 318 (12):43–47.

Hidayat, Y., Fuad, F., & Nurhidayati, M. (2018). Implementation of economic democracy principle in Islamic banking policies through Financial Services Authority (FSA) in Indonesia. **At-Taradhi: J Stu Eko,** 8(2): 132–154.

Hossain, M. S., Urbi, Z., Sule, A., & Rahman, K. M. (2014). *Andrographis paniculata* (Burm. f.) Wall. ex Nees: a review of ethnobotany, phytochemistry, and pharmacology. **The Scientific World Journal,** 2014.

Hassaan, M. S., Nagar, A. G. E., Salim, H. S., Fitzsimmons, K., & El-Haroun, E. R. (2019). Nutritional mitigation of winter thermal stress in Nile tilapia by propolis-extract: Associated indicators of nutritional status, physiological responses and transcriptional response of delta-9-desaturase gene. **Aquaculture,** 511, 734256.

Husen, R., Pihie, AH.,& Nallappan, M. (2004). Screening for antihyperglycaemic activity in several local herbs of Malaysia. **J Ethnopharmacol,** 95(2- 3):205-208.

Immanuel, G., Uma, R. P., Iyapparaj, P., Citarasu, T., Punitha Peter, S. M., Michael Babu, M., & Palavesam, A. (2009). Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. **Journal of fish biology,** 74(7), 1462-1475.

Jantrarotai, W., Sitasit, P., Jantrarotai, P., Viputhanumas, T., & Srabua, P. (2007). Protein and Energy Levels for Maximum Growth, Diet Utilization, Yield of Edible Flesh, and Protein Sparing of Hybrid Clarias Catfish (*Clarias macrocephalus x C. gariepinus*). **Journal of the World Aquaculture Society,** 29(3), 281-289.

Jomard, A., & Osto, E. (2020). High density lipoproteins: metabolism, function, and therapeutic potential. **Frontiers in cardiovascular medicine,** 7, 39.

Kamaraj, C., Deepak, P., Balasubramani, G., Karthi, S., Arul, D., Aiswarya, D., & Perumal, P. (2018). Target and non–target toxicity of fern extracts against mosquito vectors and beneficial aquatic organisms. **Ecotoxicology and Environmental Safety,** 161, 221–230.

Kareem, Z.H., Abdelhadi, Y.M., Christianus, A., Karim, M., & Romano, N. (2016). Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (*Oreochromis niloticus*) and their resistance to Streptococcus agalactiae infection. **Fish Physiol Biochem,** 42:757–769.

Kartikaningsih, H., Rohman, F. Z., & Jaziri, A. A. (2020). Characteristics of *Aeromonas hydrophila* –infected Catfish (*Clarias sp.*). **In IOP Conference Series: Earth and Environmental Science,** (Vol. 493, No. 1, p. 012036). IOP Publishing.

Khunchalee, J., & Munglue, P. (2020). Effects of Cardamonin Enriched Diets on Growth, Intestinal Histology, Hematology, and Biochemical Parameters of Hybrid Catfish (*Clarias macrocephalus× Clarias gariepinus*). **CMU J. Nat,** 19 (4) 901. https://doi.org/10.12982/CMUJNS.2020.0056

Kokate, C.K. (1994). Practical Pharmacognosy, 4th ed., Vallabh Prakasan, Delhi, 107–111.

Ko, H.C., Wei, B.L., & Chiou, W.F. (2006). The effect of medicinal plants used in Chinese folk medicine on RANTES secretion by virus infected human epithelial cells. **J Ethnopharmacol,** 107:205–210.

Koskela, J., Rahkonen, R., Pasternack, M., & Knuutinen, H. (2004). Effect of immunization with two commercial vaccines on feed intake, growth, and lysozyme activity in European whitefish (*Coregonus lavaretus L.*). **Aquaculture,** 234(1–4), 41–50.

Lee, K. W., Everts, H., Kapperst, H. J., Yeom, K. H., & Beynen, A. C. (2003). Dietary carvacrol lowers body weight gain but improves feed conversion in female broiler chickens. **Journal of Applied Poultry Research,** 12(4), 394–399.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. **Molecules,** 21(10), 1374.

Lin, T.P., Chen, SY., Duh, P.D., Chang, L.K., & Liu, Y.N. (2008). Inhibition of the Epstein–barr virus lytic cycle by andrographolide. **Biol Pharm Bull,** 31(11):2018–2023.

Lucas, A. (1996). Physical concepts of bioenergetics. **Bioenergetics of aquatic animals. English edition,** Taylor and Francis, France.

Maiti, S., Saha, S., Jara, P., Chowdhury, A., Khatua, S., & Ghosh, T.K. (2021). Effect of dietary *Andrographis paniculata* leaf extract on growth, immunity, and disease resistance against *Aeromonas hydrophila* in *Pangasianodon hypopthalmus.* **journal of applied aquaculture,** 1–25.

Mishra, A., Shah, B. R., Roy, K., Abdelsalam, E. E. E., Pia**Č**ková, V., Shaik, H. A., & Mráz, J. (2023). Andrographolide loaded Pickering emulsion: A bioactive component for improved growth, digestibility, and haematological properties in cultured common carp *Cyprinus carpio*. **Aquaculture,** 562, 738810.

Munglue, P., Rattana, K., Sangchanjiradet, S., Jankam, A., & Dasri, K. (2019). Growth performance and intestinal morphology of hybrid catfish *(Clarias macrocephalus× Clarias gariepinus*) fed diet supplemented with rice paddy herb (*Limnophila aromatica*) extract. **Asia–Pacific Journal of Science and Technology,** 24(2).

Murtola, T., Vuorela, T. A., Hyvönen, M. T., Marrink, S. J., Karttunen, M., & Vattulainen, I. (2011). Low density lipoprotein: structure, dynamics, and interactions of apoB–100 with lipids. **Soft Matter,** 7(18), 8135–8141.

Nagajothi, S., Mekala, P., Raja, A., Raja, M. J., & Senthilkumar, P. (2018). *Andrographis paniculata*: qualitative and quantitative phytochemical analysis. **Journal of Pharmacognosy and Phytochemistry,** 7(4), 1251–1253.

Najila, M. S., Rain, A. N., Kamel, A. M., Zahir, S. S., Khozirah, S., Hakim, S. L., & Azizol, A. K (2002). The screening of extracts from *Goniothalamus scortechinii*, *Aralidium pinnatifidum* and *Andrographis paniculata* for anti–malarial activity using the lactate dehydrogenase assay. **Journal of Ethnopharmacology,** 82(2–3), 239–242.

Naomi, R., Bahari, H., Ong, Z. Y., Keong, Y. Y., Embong, H., Rajandram, R., & Zakaria, Z. A. (2022). Mechanisms of Natural Extracts of *Andrographis paniculata* That Target Lipid–Dependent Cancer Pathways: A View from the Signaling

Pathway. **International Journal of Molecular Sciences,** 23(11), 5972.

Ng, W. K., & Chen, M. L. (2002). Replacement of soybean meal with palm kernel meal in practical diets for hybrid Asian–African catfish, *Clarias macrocephalus*× *C. gariepinus*. **Journal of Applied Aquaculture,** 12(4), 67–76.

Nya, E. J., & Austin, B. (2009). Use of garlic, Allium sativum, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). **Journal of fish diseases,** 32(11), 963–970.

Palanikani, R., Soranam, R., & Chanthini, K. M. P. (2018). Pathogenicity and control of *Aeromonas hydrophila* and *A. veronii* in Indian major carps (*Catla–catla*) by the effect of herbal supplement of *Andrographis paniculata* (*Lamiales: Acanthaceae*). **Inter J Fish Aqu Stud,** 8(3), 361–370.

Palanikani, R., Chanthini, K. M. P., Soranam, R., Thanigaivel, A., Karthi, S., Senthil–Nathan, S., & Murugesan, A. G. (2020). Efficacy of *Andrographis paniculata* supplements induce a non–specific immune system against the pathogenicity of *Aeromonas hydrophila* infection in Indian major carp (*Labeo rohita*). **Environmental Science and Pollution Research**, 27, 23420–23436.

Pandey, A. K., & Mandal, A. K. (2010). Variation in morphological characteristics and andrographolide content in *Andrographis paniculata* (Burm. f.) Nees of Central India. **Iranica J Energy Environ,** 1(2), 165–169.

Panase, P., Kamee, B., Moungmor, S., Tipdacho, P., Matidtor, J., & Sutthi, N. (2018a). Effects of *Euphorbia hirta* plant leaf extract on growth performance, hematological and organosomatic indices of hybrid catfish, *Clarias macrocephalus*× *C. gariepinus*. **Fisheries science,** 84, 1025–1036.

Panase, P., Khuangbun, L., Suphason, T., & Tipdacho, P. (2018b). Evaluation of *Houttuynia cordata Thunb*. leaf extract on growth performance, feed utilization, and hematological indices of hybrid catfish (*Clarias macrocephalus*× *Clarias gariepinus*). **Comparative Clinical Pathology,** 27, 947–958.

Peng, K., Zhao, H., Wang, G., Chen, B., Mo, W., & Huang, Y. (2021). Effect of condensed tannins on growth performance, intestinal immune capacity and bacterial microbiomes of *Lateolabrax japonicus*. **Aquaculture Research,** 52(11), 5321–5331.

Phommanivong, S.,&Doolchidachabaporn, S. (2013). Effects of Moringa's leave supplementary diet on growth performances and survival rate of redtail mystus (Hemibagrus wyckioides). **Journal of Fisheries Technology Research,** 7(1), 9–19.

Pourmoghim, H., Haghighi, M., & Rohani, M. S. (2015). Effect of dietary inclusion of Origanum vulgare extract on non–specific immune responses and hematological parameters of rainbow trout (*Oncorhynchus mykiss*). **Bulletin of environment, pharmacology and life sciences,** 4(3), 33–39.

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(3), 240–251.

Puri, A., Saxena, R., Saxena, R.P., Saxena, K.C., Srivastava, V., & Tandon, J.S. (1993) Immunostimulant agents from *Andrographis paniculata*. **J Nat Prod,** 1993, 56(7):995–999.

Quesada, S. P., Paschoal, J. A. R., & Reyes, F. G. R. (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—a review. **Journal of food science,** 78(9), R1321–R1333.

Rajalakshmi, V., & Cathrine, L. (2016). Phytochemical screening and antimicrobial activity of ethanolic extract of *Andrographis paniculata*. **Journal of Pharmacognosy and Phytochemistry**, 5(2), 175–177.

Rajalakshmi, V., & Cathrine L. (2016). Phytochemical screening and antimicrobial activity of ethanolic extract of *Andrographis paniculata*. **Journal of Pharmacognosy and Phytochemistry,** 5 (2), 175–177. doi: 10.4103/0250–474X.57294.

Rauf, A., Jan, M., Rehman, W., & Muhammad, N. (2013). Phytochemical, phytotoxic and antioxidant profile of *Caralluma tuberculata* NE Brown. **Wudpecker Journal of Pharmacy and Pharmacology,** 2(2), 21–25.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2009). Prophylactic effect of *Andrographis paniculata* extracts against Streptococcus agalactiae infection in Nile tilapia (Oreochromis niloticus). **Journal of bioscience and bioengineering,** 107(5), 579–582.

Rehulka, J. (2003). Haematological analyses in rainbow trout *Oncorhynchus mykiss* affected

by viral haemorrhagic septicaemia (VHS). **Diseases of aquatic organisms,** 56(3), 185–193.

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. https://doi.org/10.1016/j.aquaculture.2014.05.048

Ronald, W.G., & Bruce, A.B. (1990). Organosomatic indices and an autopsy–based assessment as indicators of health condition of fish. **J Am Fish Soc,** 8, 93–108. doi: 10.4236/ijg.2012.33049.

Roongkamnertwongsa, J. and Roongkamnertwongsa, S. (2010). **Effects of crude extract *Andrographis paniculata* on blood component,** immune system and disease resistance in seabass (Lates calcarifer Bloch, 1790). Warasan Kan Pramong.

Rudy, A.N., Meylianawati, O.F.A., Yanti, P.S., & Esti, H.H. (2018). The effects of dietary *Eleutherine bulbosa* on the growth, leukocyte profile, and digestive enzyme activity of the striped catfish *Pangasianodon hypophthalmus*. **Nusant. Biosci. 10,** 46–51

Shalini, V. B. & Narayanan, J. S. (2015). Characterization studies on medicinal plant of *Andrographis paniculata* (NEES). **Journal of Medicinal Plants Studies,** 3(5), 96–102.

Sharon, L. A., Wendy, K.W. C., & Janda J. M. (2003). The Genus *Aeromonas*: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes. **JOURNAL OF CLINICAL MICROBIOLOGY,** June 2003, p. 2348–2357.

Sharma, M., and Sharma, R. (2013). Identification, purification and quantification of andrographolide from *Andrographis paniculata* (burm. F.) Nees by HPTLC at different stages of life cycle of crop. **J curr chem pharm sci,** 3(1), 23–32

Sheikhlar, A., Alimon, A. R., Daud, H., Saad, C. R., Webster, C. D., & Meng, G. Y. (2014). White mulberry (*Morus alba*) foliage methanolic extract can alleviate *Aeromonas hydrophila* infection in African Catfish (*Clarias gariepinus*). **The Scientific World Journal,** 2014.

Sheikhlar, A., Meng, G. Y., Alimon, R., Romano, N., & Ebrahimi, M. (2017). Dietary *Euphorbia hirta* extract improved the resistance of sharptooth catfish

Clarias gariepinus to *Aeromonas hydrophila*. **Journal of aquatic animal health,** 29(4), 225–235.

Sheeba, S. (2012). **Effect of oral immunostimulant *Andrographis paniculata* and resistance to *Aeromonas hydrophila* in *Catla catla*.** Innocent Xavier B et al / IJRAP, 3(2).

Sheeja, K., Guruvayoorappan, C., & Kuttan G. (2007). Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. **Int Immunopharmacol,** 7:211–221.

Sheeja, K., & Kuttan, G. (2007). Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by *Andrographis paniculata* extract and andrographolide. **Immunopharmacol Immunotoxicol,** 29:81–93.

Shi, Y., Zhong, L., Liu, Y., Zhang, J., Lv, Z., Li, Y., & Hu, Y. (2020). Effects of dietary andrographolide levels on growth performance, antioxidant capacity, intestinal immune function and microbioma of rice field eel (*Monopterus albus*). **Animals,** 10(10), 1744.

Singh, R.P., Banerjee, S., & Rao, A.R. (2001). Modulatory influence of *Andrographis paniculata* on mouse hepatic and extrahepatic carcinogen metabolizing enzymes and antioxidant status. **Phytother Res,** 15:382–390.

Sivananthan, M., & Elamaran, M. (2013). Medicinal and pharmacological properties of *Andrographis paniculata*. **Int. J. Biomol. Biomed, 3,** 1–12.

Suriyo, T., Chotirat, S., Rangkadilok, N., Pholphana, N., & Satayavivad, J. (2021). Interactive effects of *Andrographis paniculata* extracts and cancer chemotherapeutic 5–Fluorouracil on cytochrome P450s expression in human hepatocellular carcinoma HepG2 cells. **J Herb Med** 26:100421. https://doi.org/10.1016/j.hermed. 2021.100421

Sutthi, N., Panase, A., Chitmanat, C., Sookying, S., Ratworawong, K., & Panase, P. (2020). **Effects of dietary leaf ethanolic extract of *Apium graveolens L.* on growth performance, serum biochemical indices, bacterial resistance and lysozyme activity in *Labeo chrysophekadion* (Bleeker, 1849).** Aquaculture Reports, 18, 100551.

Tan, X., Sun, Z., Chen, S., Chen, S., Huang, Z., Zhou, C., & Wang, A. (2017). Effects of

dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano *Trachinotus ovatus*. **Fish & Shellfish Immunology,** 66, 198–206.

Valdiani, A., Talei, D., Lattoo, S.K., Ortiz, R., Rasmussen, S.K., Batley, J., Rafi, M.Y., Maziah, M., Sabu, K.K., Abiri, R., Sakuanrungsirikul, S., & Tan, S.G. (2017). Genoproteomics–assisted improvement of *Andrographis paniculata*: toward a promising molecular and conventional breeding platform for autogamous plants afecting the pharmaceutical industry. **Critic Rev Biotechnol** 37(6):803–816. https://doi.org/10.1080/07388551.2016.1260525

Valdiani, A., Ofoghi, H., Akbarizare, M., & Talei, D. (2022). *Andrographis paniculata* extract as an immunity modulator against cancer via telomerase inhibition. 3 **Biotech,** 12:319.https://doi.org/10.1007/s13205–022–03373–2

Velichkova, K., Sirakov, I., Stoyanova, S., Zhelyazkov, G., Staykov, Y., & Slavov, T. (2019). Effect of *Acorus calamus L.* extract on growth performance and blood parameters of common carp (*Cyprinus carpio L.*) cultivated in a recirculation system. **Journal of Central European Agriculture,** 20(2), 585–591.

Verma, N., & Vinayak, M. (2008). **Antioxidant action of *Andrographis paniculata* on lymphoma. Mol Biol Rep,** 35:535–540.

Vijayan, M. M., Pereira, C., Forsyth, R. B., Kennedy, C. J., & Iwama, G. K. (1997). Handling stress does not affect the expression of hepatic heat shock protein 70 and conjugation enzymes in rainbow trout treated with β–naphthoflavone. **Life sciences,** 61(2), 117–127.

Villegas, J.,& Hosokawa, H. (2004). **Immunostimulants: towards temporary prevention of diseases in marine fish.** Avances en nutricion acuicola.

Wang, C.Y., Li, Z.B., Sun, Y. Z., Chen, Q., Li, W.J., Huang, Y.C., & Lu, J. (2018). Effects of Chinese herbal medicines mixture on growth performance digestive enzyme activity immune response of juvenile Japanese seabass, ***Lateolabrax japonicus.*** **Aquaculture Nutrition,** 24, 683–693. https://doi.org/10.1111/anu.12597

Wangkahart, E. (2018). **Effect of *Aeromonas hydrophila* Infection on Hematological**

Value in Hybrid Catfish (*Clarias macrocephalus x C. gariepinus*) and Sensitivity Test to Antibiotic Drugs. SWU Sci. J.

Xu, A., Shang-Guan, J., Li, Z., Gao, Z., Huang, Y., & Chen, Q. (2020). Effects of garlic powder on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, Lateolabrax japonicus. **Aquacult Nutr.** 2020;00:1–10. https://doi.org/10.1111/anu.13001

Xu, Y., Chen, A., Fry, S., Barrow, R.A., Marshall, R.L., & Mukkur, T.K.S. (2007). Modulation of immune response in mice immunized with an inactivated Salmonella vaccine and gavaged with *Andrographis paniculata* extract or andrographolide.**Int Immunopharmacol 2007,** 7:515–5238.

Zhang, XF.,& Tan, B.K. (2000). Antihyperglycaemic and antioxidant properties of *Andrographis paniculata* in normal and diabetic rats. **Clin Exp Pharmacol Physiol,** 27:358–363.

Zhang, CY., & Tan, B.K. (1996). Hypotensive activity of aqueous extract of *Andrographis paniculata* in rats. **Clin Exp Pharmacol Physiol,** 23:675–678.

Zheng, Z. L., Tan, J. Y., Liu, H. Y., Zhou, X. H., Xiang, X., & Wang, K. Y. (2009). Evaluation of oregano essential oil (*Origanum heracleoticum* L.) on growth, antioxidant effect and resistance against *Aeromonas hydrophila* in channel catfish (*Ictalurus punctatus*). **Aquaculture,** 292(3–4), 214–218.

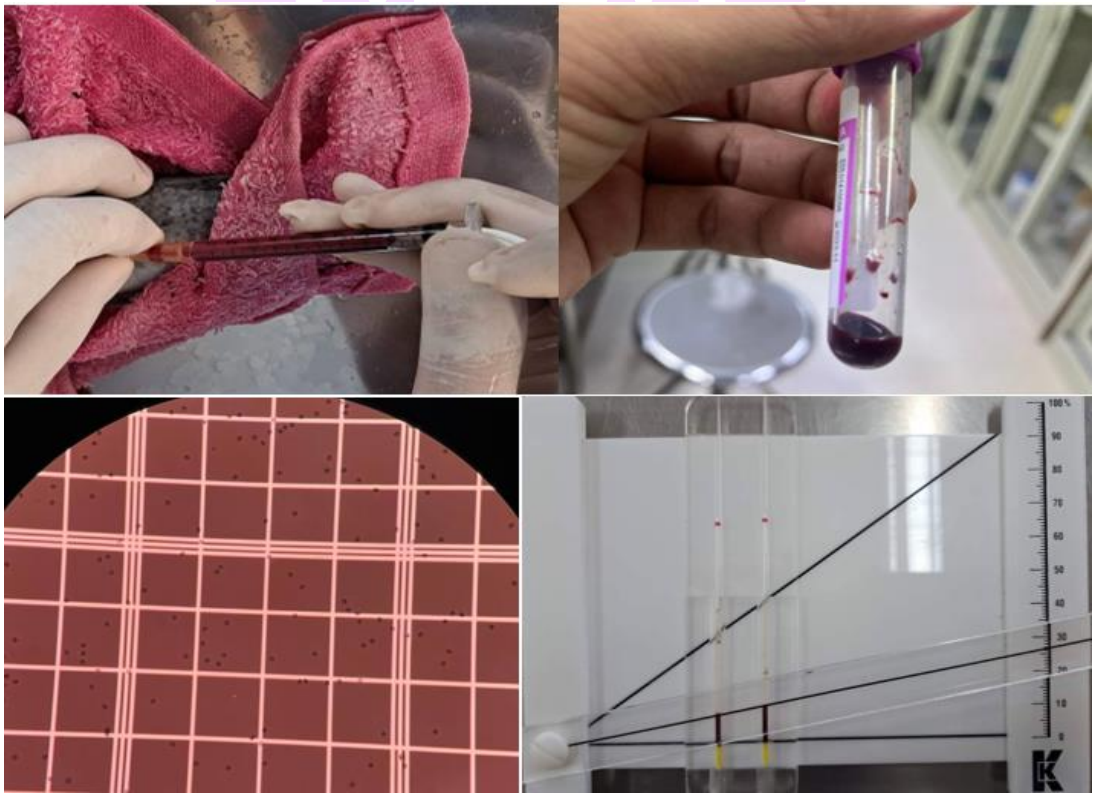
APPENDIX

Figures 36 Collecting and extracting plants.

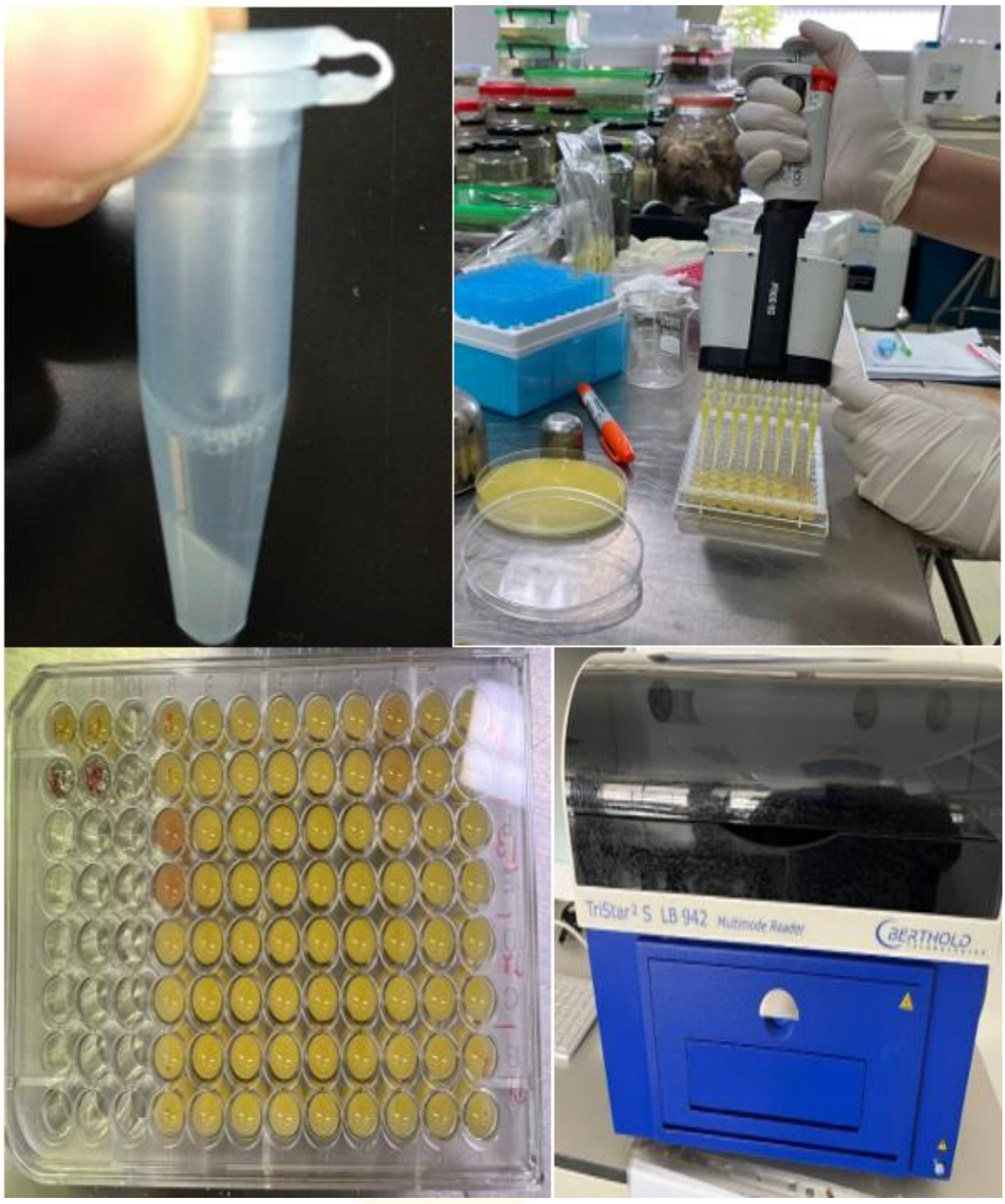
Figures 37 Procedure for mixing feed with extracts.

Figures 38 The experimental design is completely random design (CRD) and fish acclimatization.

Figures 39 At 15–day intervals, all the fish in each net cage were weighed and measured in length.

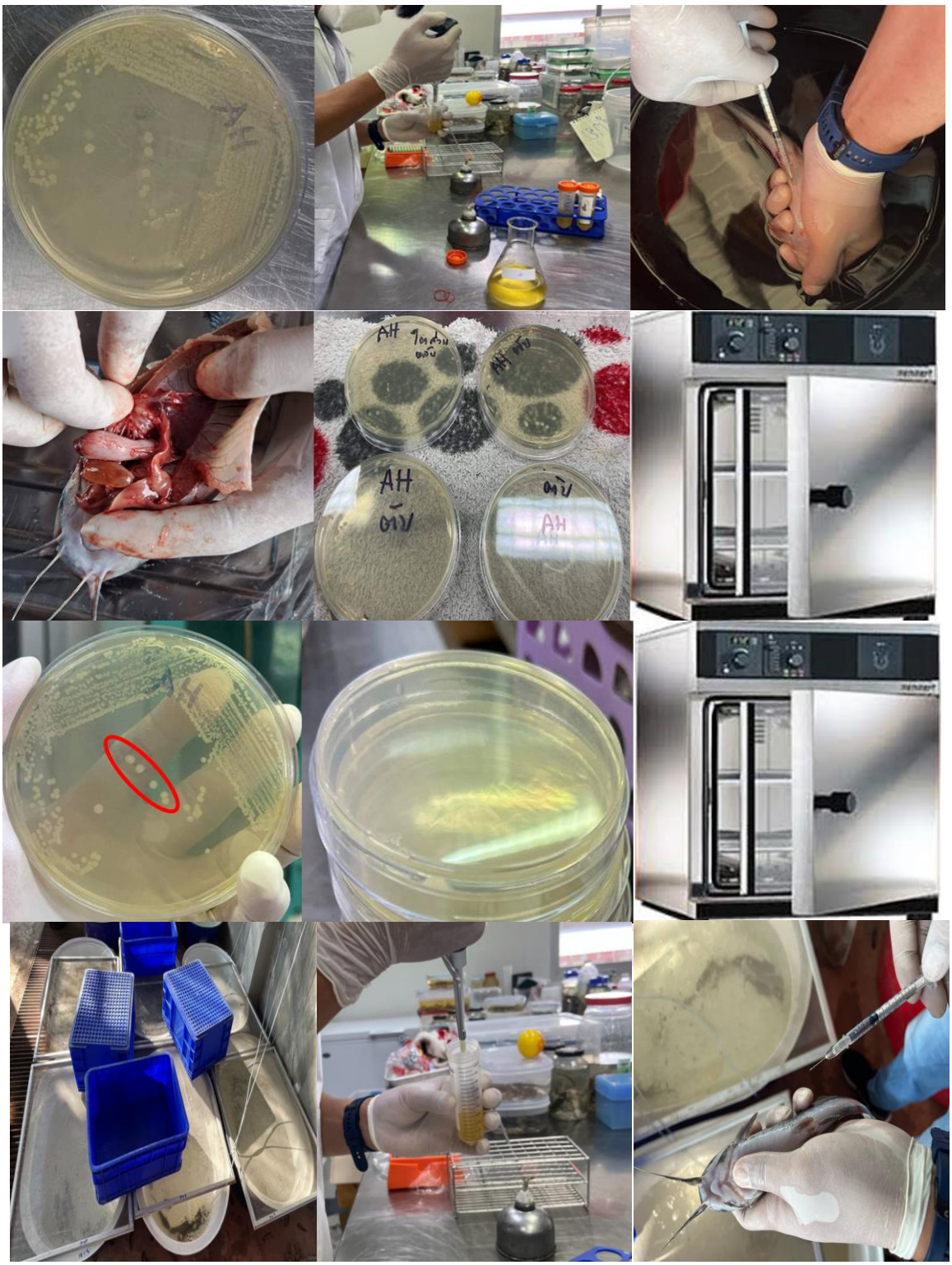


Figures 40 Blood collection and hematology value measurements



Figures 41 The procedure to do lysozyme activity.

Figures 42 The whole visceral organ was separated for the liver, spleen, kidney and intestine to calculate the hepatosomatic index (HSI,%), spleenosomatic index (SSI %), kidney (KI %) and intestinosomatic index (ISI%).



Figures 43 Preparing *Aeromonas hydrophila* and testing LD_{50} in experimental.

Figures 44 The experimental injection with *Aeromonas hydrophila* 14 days

BIOGRAPHY

NAME	Chitra Ear
DATE OF BIRTH	09.03.1994
PLACE OF BIRTH	Cambodia
INSTITUTIONS ATTENDED	Year: 2564–2566 Master of Science Program in Fisheries Technology and Innovation. University of Phayao. Year: 2556–2559 Bachelor of fisheries science on Rajamangala University of technology Isan Sakon Nakhon campus.
HOME ADDRESS	Rajamangala University of Technology Isan, (RMUTI),Sakon Nakhon Campus Thailand
PUBLICATION	Chitra Eara,b,c, Sontaya Sookyingc,d, Korntip Kannikaa,c, Chatmongkon Suwannapoomaand Paiboon Panase.(2024). Positive effects ofAndrographis paniculataextract on growth performance,haematology, serum biochemistry, organosomatic indices and resistance againstAeromonas hydrophilain hybrid catfish (Clarias macrocephalus × Clarias gariepinus). Journal of Applied Animal Research Volume 52, 2024 – Issue 1. NO. 1, 2320230https://doi.org/10.1080/09712119.2024.2320230