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Effects of guava (*Psidium guajava* L.) and bhumi amla (*Phyllanthus amarus* Chum et Thonn) extracts on haematological parameters and oxidative stress of striped catfish (*Pangasianodon hypophthalmus*) fingerlings exposed to high-temperature stress

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ABSTRACT

Effects of guava (Psidium guajava L.) and bhumi amla (Phyllanthus amarus Chum et Thonn) on haematology and thermal stress mitigation of striped catfish (Pangasianodon hypophthalmus) were investigated. In a 42-day trial, fish were administered 4 diets as control (without extract), 0.2% P. guajava (Pg0.2), 0.5% P. amarus (Pa0.5), and a mixture of Pg0.2 and Pa0.5 (Mix). Fish were then subjected to temperatures of 27°C, 31°C, and 35°C for another 42 days. Haematological parameters were highest at 35°C, but these parameters were not significantly different from values recorded at 31°C on day 14 post-temperature challenge. The Pg0.2 diet modified red blood cells, haematocrit, and haemoglobin ($p<0.05$). The lowest glucose concentration was recorded in Pg0.2 (57.4±1.34 mg/100 mL) and Mix (58.9±1.87 mg/100 mL) groups after 14 days of thermal exposure. Glucose concentration surged on the third-day post-temperature challenge, then declined, and was maintained at 35°C until the end of the experiment which was not significant if compared to those at 27°C. Pg0.2 and Mix diets significantly reduced lipid peroxidation and enhanced catalase in gill and liver after 42 days. In the case average water temperature in the Mekong Delta remains below 35°C, the feeding diets for P. hypophthalmus administered Pg0.2 or Mix (Pg0.2+Pa0.5) extracts improve fish health through haematology and oxidative stress resistance.

1. INTRODUCTION

Striped catfish (*Pangasianodon hypophthalmus*) in the Mekong Delta, Vietnam is one of the most crucial species with an export turnover of more than $1.62 billion and total production of 1.52 million tons in 2021 (MARD, 2022). However, the production and export of *P. hypophthalmus* have encountered a variety of obstacles in farming and processing which remain inconsistent and complex to manage, with one of the most impactful obstacles being the direct impact of climate change. Over the last five decades, the average temperature in Vietnam has climbed roughly 0.5-0.7°C; and by the end of the 21st century, the annual average temperature of Vietnam is predicted to reach about 2.3°C (MONRE, 2009). Furthermore, assessments by IPCC (2013) predict the temperature to increase

by another 1-4°C in the following century. As fish are poikilotherms, it is crucial to understand their responses to variations in ambient temperature (Fry, 1971). The repercussions of the temperature increase associated with climate change are believed to be multifaceted. Most fishes are able to adjust to slight temperature alterations, but significant shifts might negatively impact growth performance, survival rate, and trigger adverse changes in biological response. This is particularly troublesome for ectotherms that have limited potential to intrinsically modulate their body temperature (Imsland et al., 2007; Portner et al., 2001; Kemp, 2009; Wright and Tobin, 2011). The most frequent effect involves stress which is associated with the variability in water temperature generated by a daily shift or seasonal change (Ju et al., 2002). Any biological, physical, or chemical stimulus that causes physiological reactions is referred to as stress (Soltanian et al., 2014), The reallocation of energy away from non-essential biological mechanisms and toward assisting in the restoration of homeostasis is a crucial component of the response to stress (Fuzzen et al., 2011). Homeostasis can be restored without severe consequences if an animal can adapt to a stressor efficiently through adaptation or if the stressor is temporary (Barton et al., 2002). Under intensive fish farming conditions, disturbances are frequently prolonged, and the associated chronic stress will result in a persistent homeostasis disturbance; adaptation is impossible after a considerable long term. As the stress response changes from adaptive to maladaptive, disease resistance, and growth are eventually constrained (Van Weerd et al., 1998).

In aquatic conditions, organisms typically produce reactive oxygen species (ROS), also known as free radicals, and there is a fine balance between the generation of ROS and the mechanism that defends cells from ROS. The breakdown of this balance triggers oxidative stress (Chowdhury and Saikia, 2020). One of the effects is accelerated lipid peroxidation (LPO) as a result of the oxidation of the unsaturated fatty acids in cell membranes (Semren et al, 2018). During natural or farming conditions, modifications in temperature, oxygen, and salinity can trigger stress by inducing an imbalance between ROS production and eradication (Chowdhury and Saikia, 2020). ROS accumulation during recovery from thermal stress can be attributed to tissue re-oxygenation (Halliwell and Gutteridge, 2015) and hyperthermia (Lushchak, 2011). Through a system of enzyme antioxidants such as superoxide dismutases (SOD), catalase (CAT), and peroxidases, organisms might restrict, counteract, or eradicate these ROS. Briefly, ROS are transformed to hydroperoxide (H_2O_2) by SOD, and subsequently to oxygen and water by various enzymes such as CAT and glutathione reductase (GR) (Lesser, 2006).

The strengthening of antioxidant activity of herbal extracts in fish is attributed to specific phytochemicals revealed in plant extracts including polyphenols (gallic acid, ellagic acid, and tannins), enzymes (SOD, CAT…), flavonoids (flavones, isoflavones, catechins, and anthocyanins) and vitamins (C, E, and carotenoids) (Gupta and Sharma, 2014). Moreover, the application of phyto-additives has been known to promote a variety of critical biochemical functions in fish by helping to uptake glucose to relieve stress and improving haematological parameters via the anti-anemic adverse effect (Oh et al., 2005; Shen et al., 2008; Cheng et al., 2009; Adeyemi et al., 2010; Shakeera et al., 2013).

Accordingly, the potency of bioactive substances in guava (*Psidium guajava* L.) and bhumi amla (*Phyllanthus amarus* Chum et Thonn.) extracts on fish were revealed in antimicrobial activity and the fish's immune response of different species (Pachanawan et al., 2008; Ferdous et al., 2017; Nhu et al., 2019; Dao et al., 2020; Nhu et al., 2020). In Vietnam, though some investigation regarding plant extracts in the veterinary medicine field has been conducted, findings on the benefits of frequently available medicinal herbs on stress mitigation as well as oxidative stress of farmed *P. hypophthalmus* are fragmentary. Additionally, there is currently minimal evidence regarding haematological parameters as well as investigating the function of the two mentioned extracts on the haematology and antioxidant activities of *P. hypophthalmus* under elevated thermal stress.

The main objective of this research evaluates the effect of *P. guajava* and *P. amarus* extracts in single and mixed forms on haematology and oxidative stress biomarkers of *P. hypophthalmus* fingerlings under thermal stress, aiming to provide relevant details for efficient farming of *P. hypophthalmus* to strengthen health, improve efficiency and thus mitigate stress in climate change scenarios.

2. MATERIALS AND METHODS

2.1. Plant extracts and diet preparation

Fresh specimens of *P. amarus* (leaves and twigs) and *P. guajava* (leaves) were obtained from Can

Tho city, Vietnam. The extracts of these plants were processed at the College of Natural Sciences, Can Tho University. The plant portions were sun-dried for three days before being dried at 60°C. The plant's ethanolic extract was prepared by immersing 100 g of dry powder in 96° ethanol (800 mL) for 1 day. The obtained compound was decanted and screened, and the residual solvent was evaporated using a low-pressure rotary evaporator (Nhu et al., 2020)

The extract was incorporated into the diet of *P. hypophthalmus* as either 0.2% *P. guajava* (Pg0.2), 0.5% *P. amarus* (Pa0.5), or a mixture of Pg0.2 and Pa0.5 (Mix). The selected levels of extracts were based on prior results regarding the effectiveness of these extracts on the innate immunity and antimicrobial activity of *P. hypophthalmus* as well as recommended (Nhu et al., 2020). Four regimens with different types of plant materials (Control, Pg0.2, Pa0.5, and Mix) were prepared. The iso-lipidic, iso-proteic, and iso-energetic diets were used in the experiments. The control diet was a basal diet with no plant extract supplement. All ingredients were thoroughly blended into a homogeneous mixture before being pelletized, air-dried, ground, and sieved to achieve the desired pellet size (2 mm). The pellets were stored at -20°C in properly labeled polythene bags.

2.2. Experimental fish acclimation, facilities, and feeding trial

P. hypophthalmus fingerlings (12.1±0.04 g/fish) were obtained from a hatchery in Can Tho city and delivered in well-oxygenated bags to the laboratory. The fish were acclimated in $2m^3$- experimental tanks with well-aerated water and a natural photoperiod. Fish were fed a basal diet twice a day (at 8:00 am and 4:00 pm) until they were satiated (3-5% of their body weight).

The experiment was administered randomly for two periods. The feeding period: a total of 2,700 healthy *P. hypophthalmus* fingerlings were uniformed and randomly assigned to 36 experimental tanks (300-L, 75 fish/tank); fish were hand-fed daily to satiation with their designated feed (corresponding to four groups including control, Pg0.2, Pa0.5 and Mix with 9 replicates) over the experimental duration of 42 days. The temperature challenge period which followed was a 3 × 4 factorial experiment designed to compare the effects of three temperatures (27°C (control), 31°C, and 35°C) with the four previously mentioned diets. Those fish in the feeding trial were acclimated to three selected temperature conditions in 4 days, then cultured for 42 days at desired temperatures. The temperature was gradually elevated (Δ1°C per 24 h for the 31°C treatment, and Δ2°C per 24 h for the 35°C treatment) from ambient temperature (27°C). A total of 36 tanks of identical sizes of fish (20.4±0.15 g, triplicated for 12 treatments) with a stocking density of 45 fish/tank. The desired temperatures were maintained using thermostats and heaters. Blood was collected from 3 fish from each tank during the temperature challenge period including 0 h, day 1, day 3, day 7, and day 14. To investigate oxidative stress indicators, the gills and liver were collected at 0 h, day 7, day 14, and 42.

Throughout the experiment, all tanks were continually aerated. Fish were manually fed twice a day until they appeared to be satiated (3-5% of their body mass). Wastes accumulated on the tank bottom were siphoned daily, and 30% of the water was exchanged weekly with fresh dechlorinated water. A pH instrument (Metler Toledo SG2, USA) was used to measure water pH twice a week, and an Oxy Guard H04PP was used to measure dissolved oxygen and temperature. Dissolved oxygen (3.65 to 4.40 mg/L), pH (6.72 to 7.29), and temperature (0.1°C) were all within the suitable range for *P. hypophthalmus*. Accordingly, the experiment was conducted in accordance with general legislation on the protection and experimental animal welfare in Viet Nam (Law of animal health, 2015).

2.3. Haematological and biochemical parameters

Three fish from each replicate (9 fish/treatment) were randomly selected from tanks at the following time intervals: blood was sampled at the time reaching the desired temperature in each treatment at 0 h, day 1, day 3, day 7, and day 14. A cool moist cloth was placed on the head of each sampled fish (Snellgrove and Alexander 2011) for minimizing stress during handling. Heparinized syringe of 1 mL was used to sample blood from the caudal vein within 30 seconds. At least 300 µL of blood from each fish were withdrawn and transferred into a labeled 1.5 mL tube.

A portion of the blood was used to identify haematological parameters. Natt and Herrick's solution was gently mixed with the diluted blood (1:200). The cell suspension was placed in a Neubauer haemocytometer and red blood cells (RBCs) were quantifed at a magnification of 40× (Natt and Herrick 1952). Haematocrit (Hct) was measured within 6 minutes at 12,000 rpm in a microhaematocrit centrifuge (Sigma 201M, Hettich)

(Larsen and Snieszko 1961). Drabkin's reagent was used to ascertain haemoglobin (Hb); 10 µL of the solution was diluted with 2.5 mL of Drabkin's reagent and spectrometric measurements were undertaken with a spectrophotometer (GENESYSTM 20, Thermo Scientific) to determine cyanmethaemoglobin formation (Zijlstra et al. 1983). The remaining portion of the blood was centrifuged at 6000 rpm for 6 minutes at 4°C. After that, the supernatant was separated into labeled Eppendorf and stored at -80°C for subsequent analysis. A standardized glucose assay was procedured to quantify glucose concentrations (Huggett and Nixon 1957).

2.4. Oxidative stress assays

Fish were segmented to separate their gills (gill filaments) and liver (~0.5 g) for assessing oxidative stress indicators after blood was sampled. Fish were kept on ice during sampling. Each labeled Eppendorf tube containing the samples was then immediately frozen at -80°C for homogenization after being rinsed with deionized water and wiped dry with filter paper. The liver and gills were thoroughly defrosted and weighed. Afterward, these organs were then homogenized 1:5 (w:v) in ice-cold 50 mM phosphate buffer (disodium hydrogen phosphate - Na_2HPO_4 and Sodium dihydrogen phosphate - NaH_2PO_4, pH=7.5). 400 µL of homogenate was used to measure lipid peroxidation (LPO). The remaining homogenate was centrifuged at 10,000 rcf for 10 min at 4°C, and the supernatant was pipetted to a new Eppendorf tube and immediately frozen (80°C) for catalase analysis (CAT).

Malondialdehyde (MDA), a product of LPO that results in the generation of free radicals, was quantified using the thiobarbituric acid reactive substances assay (TBARS). In brief, MDA interacts with thiobarbituric acid (TBA), and the reaction mixture was spectrophotometrically measured at 535 nm. LPO was quantified as nmol MDA/g tissue using a calibration curve with ascending MDA concentrations (Fatima et al., 2000). The enzyme catalase (CAT) activity assay was determined following a procedure described by Goth (1991) using 30% H_2O_2 and ammonium heptamolybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4.H_2O$ reagents) (Sigma, USA). The sample's absorbance was measured at 405 nm. Finally, the CAT activity was calculated based on the protein concentration (Bradford, 1976) and was expressed as U/min/mg protein. Protein concentration was quantified using the Bradford (1976) procedure using bovine serum albumin (BSA) as the standard.

2.5. Statistical analysis

Statistical analyses were implemented using SPSS software, version 20 (IBM Corp., Armonk, NY). The Levene test was applied to assess the homogeneity of variance among groups. A p-value of less than 5% ($p<0.05$) was regarded as significant. Means and standard errors of the means (SEM) are displayed for all data. A general linear model (fixed = temperature, feed) was applied to test for the effect of temperature, feed, and their interactions over the 42-day temperature exposure. When significant differences among treatments were observed, the Duncan post-hoc analysis was performed to determine the significance of the difference ($p<0.05$).

3. RESULTS

3.1. Effect of plant extract on *P. Hypophthalmus* haematological parameters

There was no interaction effect between temperature level and feed on several haematological parameters. After 14 days, the administration of extracts affected the RBCs count in all sampling periods, with the highest significant group of Pg0.2 followed by the Mix group. Temperature influenced the RBCs count after 24 h (or day 1) temperature exposure until day 3 with the highest RBCs observed in 31°C groups ($3.04\pm0.073\times10^6$ cells/mm^3 and $2.88\pm0.071\times10^6$ cells/mm^3, respectively, $p<0.05$). The RBC count then declined and was maintained to day 14 among temperature groups and was not significantly different compared to the control (Table 1).

Similarly, Hb and Hct were highest in the group of fish supplemented with Pg0.2 and significant difference compared to the remaining feed groups in each given sampling time ($p<0.05$). The highest Hb was recorded on day 1 to day 3 at 31°C (9.71±0.25 mg/100 mL and 9.79±0.21 mg/100 mL, respectively) (Table 2).

Table 1. RBCs (×10^6 cells/mm^3) of *P. hypophthalmus* fingerlings under various temperatures in 14 days

Treatment		0H	Day 1	Day 3	Day 7	Day 14
Control	27°C	2.50± 0.08	2.64±0.158	2.47±0.100	2.42±0.068	2.53±0.09
	31°C	2.56±0.103	2.86±0.066	2.62±0.079	2.86±0.094	2.61±0.13
	35°C	2.43±0.190	2.37±0.043	238±0.031	2.63±0.144	2.54±0.06
Pg0.2	27°C	2.97±0.105	3.09±0.139	2.93±0.092	2.90±0.067	2.99±0.07
	31°C	3.40±0.181	3.23±0.087	3.14±0.096	3.08±0.152	3.15±0.08
	35°C	2.89±0.041	2.81±0.014	2.85±0.088	3.00±0.147	3.05±0.16
Pa0.5	27°C	2.70±0.097	2.95±0.034	2.73±0.177	2.62±0.118	2.84±0.06
	31°C	2.70±0.191	3.04±0.059	2.86±0.025	2.72±0.089	2.90±0.12
	35°C	2.66±0.056	2.58±0.083	2.56±0.119	2.52±0.183	2.83±0.16
Mix.	27°C	2.80±0.170	2.90±0.126	2.87±0.091	2.64±0.115	2.75±0.15
	31°C	2.82±0.075	3.04±0.079	2.90±0.086	2.92±0.092	2.88±0.08
	35°C	2.77±0.033	2.61±0.143	2.60±0.090	2.82±0.122	2.86±0.13
Feed	Control	2.50±0.12^a	2.62±0.09^a	2.49±0.07^a	2.58±0.10a	2.56±0.09^a
	Pg0.2	2.97±0.11^c	3.05±0.08^c	2.98±0.09^c	2.99±0.12c	3.06±0.10^c
	Pa0.5	2.69±0.12^{ab}	2.86±0.06^b	2.71±0.11^b	2.62±0.13ab	2.86±0.11^b
	Mix	2.80±0.09^{bc}	2.85±0.12^b	2.79±0.09^b	2.79±0.11bc	2.83±0.12^b
T°	27°C	2.74±0.11	2.89±0.11^B	2.75±0.12^B	2.65±0.09	2.78±0.09
	31°C	2.78±0.14	3.04±0.07^C	2.88±0.07^C	2.85±0.11	2.88±0.09
	35°C	2.69±0.08	2.59±0.07^A	2.60±0.08^A	2.74±0.15	2.82±0.13
P-value	Feed	0.001	<0.001	<0.001	0.001	<0.001
	T°	ns.	<0.001	0.002	ns.	ns.
	Feed×T°	ns.	ns.	ns.	ns.	ns.

Notes: The values on the table are the mean ± standard error (SE). In each given sampling time, different capitalized letters (A, B, C) present significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b, c) show significant differences among feed groups (p<0.05). A one-way ANOVA followed by Duncan post-hoc was used to identify significant differences.

Table 2. Haemoglobin (mg/100 mL) of *P. hypophthalmus* fingerlings under various temperatures in 14 days

Treatment		0H	Day 1	Day 3	Day 7	Day 14
Control	27°C	8.30 ±0.13	8.57±0.28	8.69±0.21	8.49±0.18	8.67±0.14
	31°C	8.78±0.24	9.04±0.15	9.43±0.19	8.79±0.19	8.73±0.23
	35°C	8.54±0.25	7.89±0.05	8.27±0.27	8.49±0.17	8.40±0.19
Pg0.2	27°C	9.61±0.32	9.90±0.24	10.0±0.11	9.87±0.30	9.95±0.04
	31°C	9.89±0.82	10.5±032	10.4±0.27	10.1±0.25	10.4±0.26
	35°C	9.77±0.30	9.33±0.24	9.39±0.01	9.89±0.31	10.1±0.24
Pa0.5	27°C	9.08±0.19	9.21±0.26	9.34±0.20	8.95±0.11	9.09±0.23
	31°C	9.18±0.10	9.56±0.22	9.46±0.23	9.19±0.34	9.14±0.08
	35°C	9.06±0.14	8.41±0.06	8.80±0.24	8.81±0.19	8.87±0.06
Mix.	27°C	9.55±0.15	9.34±0.31	9.48±0.13	9.37±0.12	9.43±0.27
	31°C	9.52±0.22	9.77±0.29	9.90±0.14	9.66±0.11	9.81±0.04
	35°C	9.35±0.23	8.52±0.29	9.02±0.11	9.17±0.20	9.63±0.24
Feed	Control	8.54±0.20^a	8.50±0.16^a	8.80±0.23	8.59±0.18^a	8.60±0.19^a
	Pg0.2	9.76±0.24^c	9.91±0.27^c	9.92±0.13^c	9.94±0.29^d	10.1±0.18^d
	Pa0.5	9.11±0.15^b	9.06±0.18^b	9.20±0.22^b	8.98±0.22^b	9.03±0.12^b
	Mix.	9.47±0.20^c	9.21±0.29^b	9.46±0.13^b	9.40±0.14^c	9.62±0.18^c
T°	27°C	9.14±0.19	9.26±0.27^B	9.38±0.16^B	9.17±0.18^B	9.29±0.17
	31°C	9.34±0.16	9.71±0.25^C	9.79±0.21^C	9.42±0.22^B	9.51±0.15
	35°C	9.18±0.23	8.54±0.16^A	8.87±0.16^A	9.09±0.22^A	9.25±0.18
P-value	Feed	<0.001	<0.001	<0.001	<0.001	<0.001
	T°	ns.	<0.001	<0.001	ns.	ns.
	Feed×T°	ns.	ns.	ns.	ns.	ns.

Notes: The values on the table are the mean ± standard error (SE). In each given sampling time, different capitalized letters (A, B, C) present significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b, c) show significant differences among feed groups (p<0.05). A one-way ANOVA followed by Duncan post-hoc was used to identify significant differences.

Table 3. Haematocrit (%) of *P. hypophthalmus* fingerlings under various temperatures in 14 days

Treatment		0 h	Day 1	Day 3	Day 7	Day 14
Control	27°C	28.6±0.56	28.2±1.46	29.7±1.31	28.1±0.38	27.3±1.52
	31°C	27.8±1.65	29.8±0.67	29.3±0.49	30.1±0.39	28.4±1.50
	35°C	27.9±1.42	25.8±1.01	26.0±0.46	29.2±0.69	28.9±0.35
Pg0.2	27°C	32.9±0.89	32.9±2.93	31.7±1.65	33.4±0.46	32.5±1.13
	31°C	32.0±1.81	31.9±0.33	33.3±0.79	34.8±0.49	33.9±1.23
	35°C	31.4±1.10	28.5±1.86	29.7±1.54	32.0±0.52	34.1±1.22
Pa0.5	27°C	30.0±1.66	30.1±1.25	31.3±0.39	32.7±1.14	31.0±0.88
	31°C	29.0±0.28	29.8±0.67	30.8±1.48	32.1±0.39	29.5±0.56
	35°C	29.9±1.63	27.1±1.18	28.3±0.86	31.9±1.02	31.4±1.01
Mix.	27°C	30.3±1.28	31.4±0.28	31.7±1.65	32.9±1.05	32.5±0.33
	31°C	31.4±0.69	31.5±0.77	33.3±0.79	34.1±0.59	33.5±0.63
	35°C	30.4±0.88	28.4±1.62	28.9±1.87	32.0±1.24	32.9±1.22
Feed	Control	28.1±1.21^{a}	27.9±1.04^{a}	28.3±0.75^{a}	29.1±0.49^{a}	28.2±1.13^{a}
	Pg0.2	32.1±1.27^{c}	31.1±1.65^{b}	31.6±1.33^{b}	33.4±0.49^{b}	33.5±1.19^{c}
	Pa0.5	29.6±1.19^{ab}	29.0±1.03^{ab}	30.1±0.85^{ab}	32.3±0.85^{b}	30.7±0.81^{b}
	Mix	30.7±0.95^{bc}	30.4±0.89^{b}	31.3±1.43^{b}	33.0±0.96^{b}	33.0±0.73^{c}
T°	27°C	30.5±1.10	30.6±1.48^{B}	31.1±1.25^{B}	31.8±0.76^{AB}	30.8±0.97
	31°C	30.0±1.10	30.8±0.61^{B}	31.7±0.89^{B}	32.8±0.47^{B}	31.3±0.98
	35°C	29.9±1.25	27.5±1.37^{A}	28.2±1.14^{A}	31.3±0.87^{A}	31.8±0.95
P-value	Feed	0.005	0.038	0.013	<0.001	<0.001
	T°	ns.	0.003	0.001	0.029	ns.
	Feed×T°	ns.	ns.	ns.	ns.	ns.

Notes: The values on the table are the mean ± standard error (SE). In each given sampling time, different capitalized letters (A, B, C) present significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b, c) show significant differences among feed groups (p<0.05). A one-way ANOVA followed by Duncan post-hoc was used to identify significant differences.

Table 4. Glucose concentration (mg/100 mL) of *P. hypophthalmus* fingerlings under various temperatures in 14 days

Treatment		0 h	Day 1	Day 3	Day 7	Day 14
Control	27°C	64.6±3.34	65.1±1.65	67.1±4.81	62.8±2.56	62.7±3.56
	31°C	64.0±2.80	71.0±3.87	69.9±2.98	65.3±2.96	65.4±1.93
	35°C	66.6±3.08	80.4±4.54	77.1±3.60	70.3±3.51	67.3±2.63
Pg0.2	27°C	55.9±1.05	59.2±3.02	59.0±3.68	57.6±2.55	58.6±1.05
	31°C	54.0±2.49	63.0±1.91	62.8±4.35	60.0±1.08	56.3±0.63
	35°C	59.6±2.54	71.9±2.65	68.7±0.61	62.5±3.82	57.3±2.34
Pa0.5	27°C	57.8±1.13	59.7±0.844	62.6±1.66	60.3±1.91	60.2±2.85
	31°C	59.3±1.51	64.0±1.25	68.4±4.33	62.1±1.74	61.0±1.21
	35°C	59.7±2.03	70.8±3.60	72.0±2.11	65.9±2.61	63.1±2.41
Mix.	27°C	57.0±1.41	61.7±3.55	60.9±0.86	60.0±0.81	58.7±1.05
	31°C	57.7±3.52	63.1±3.67	65.2±1.07	60.5±0.59	57.5±1.81
	35°C	59.2±2.24	69.1±2.96	68.0±1.29	63.6±2.38	60.5±2.74
Feed	Control	65.1±3.07^{b}	72.2±3.35^{b}	71.4±3.80^{b}	66.1±3.01^{b}	65.1±2.71^{c}
	Pg0.2	56.5±2.03^{a}	64.7±2.52^{a}	63.5±2.88^{a}	60.0±2.48^{a}	57.4±1.34^{a}
	Pa0.5	58.9±1.56^{a}	64.8±1.90^{a}	67.7±2.70^{ab}	62.8±2.09^{ab}	61.4±2.16^{b}
	Mix.	57.9±2.39^{a}	64.7±3.39^{a}	64.7±1.07^{a}	61.4±1.26^{a}	58.9±1.87^{ab}
T°	27°C	58.8±1.73	61.4±2.27^{A}	62.4±2.75^{A}	60.2±1.96^{A}	60.0±2.13
	31°C	58.8±2.58	65.3±2.67^{A}	66.6±3.18^{A}	62.0±1.59^{A}	60.1±1.4
	35°C	61.3±2.47	73.1±3.44^{B}	71.4±1.90^{B}	65.6±3.08^{B}	62.0±2.53
P-value	Feed	0.001	0.011	0.017	0.030	0.001
	T°	ns.	<0.001	0.001	0.014	ns.
	Feed×T°	ns.	ns.	ns.	ns.	ns.

Notes: The values on the table are the mean ± standard error (SE). In each given sampling time, different capitalized letters (A, B, C) present significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b, c) show significant differences among feed groups (p<0.05). A one-way ANOVA followed by Duncan post-hoc was used to identify significant differences.

Moreover, the lowest increase in Hct was obtained from 24 h (27.5±1.37%) exposure to high temperature (35°C) until day 7 (31.3±0.87%) and is significantly different from the remaining temperature groups (p<0.05); Hct then proceeded to decrease (p>0.05) (Table 3).

The changes in plasma glucose concentrations of fish are presented in Table 4. The Pg0.2 group obtained the lowest blood glucose concentration followed by Mix and a significant difference compared to the remaining diet in each sampling period. Notably, the fish attained the highest concentration of glucose at 35°C and a significant difference from the lower temperature groups from day 1 to day 7. After 24h from the target temperature time (day 1), glucose concentration soared, then declined, and remained relatively stable until the end of the trial.

3.2. Effect of plant extract on *P. hypophthalmus* oxidative stress

During the 42-day high-temperature exposure, no interaction between temperature and feed was observed in terms of oxidative stress biomarkers (LPO and CAT in gills and liver). The temperature had no significant effect on LPO values in the liver until after day 14 (p<0.05). Moreover, the LPO values in gills remained higher after day 7 of temperature exposure. The temperature had no significant effect (p>0.05) on LPO in any of the tested tissues on day 42 (Fig. 1 A-B).

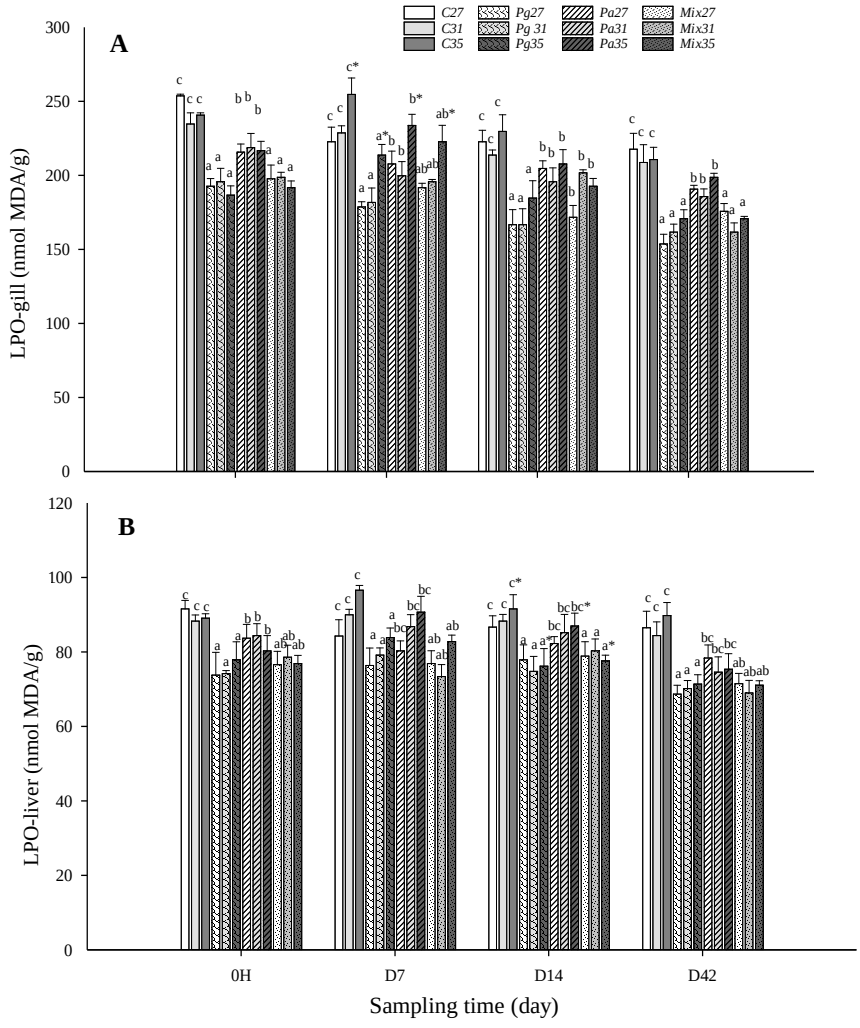

Figure 1. LPO liver (A) and LPO gill (B) of *P. hypophthalmus* fingerlings under various temperatures in 42 days. Asterisk (*) presents significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b) indicate significant differences among feed groups (p<0.05).

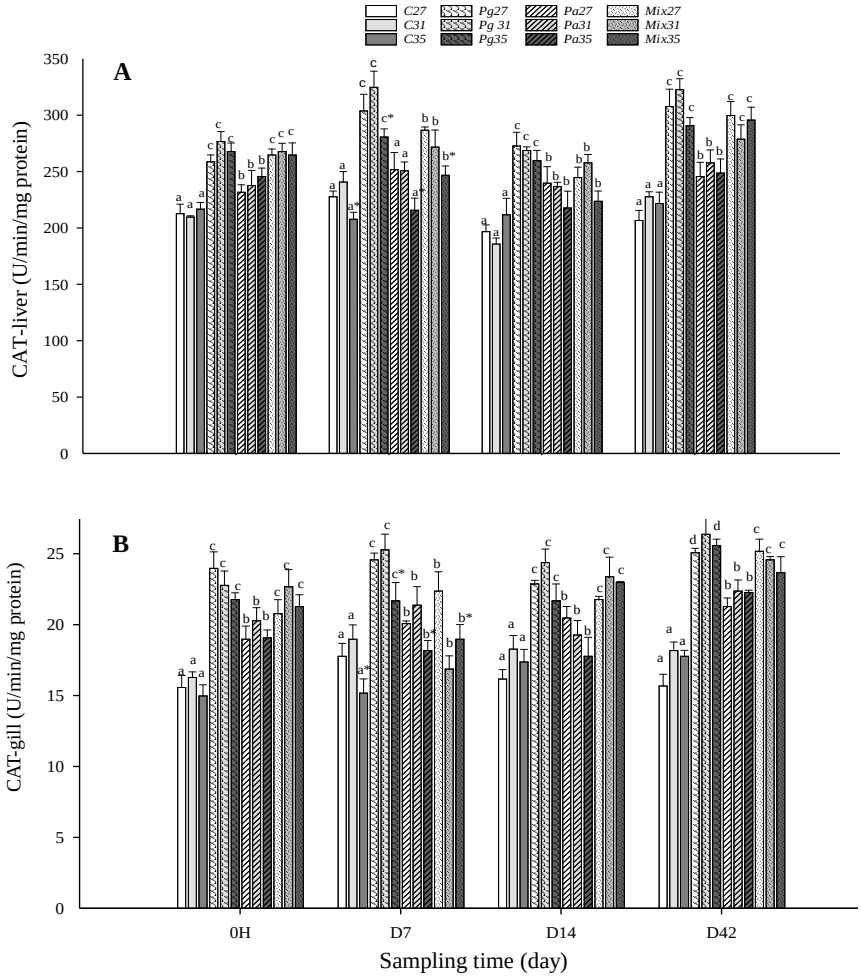

Figure 2. CAT liver (A) and CAT gill (B) of *P. hypophthalmus* fingerlings under various temperatures in 42 days. Asterisk (*) presents significant differences (p<0.05) among temperature levels whereas various lowercase letters (a, b) indicate significant differences among feed groups (p<0.05).

Regarding the effects of dietary extracts, the Pg0.2 diet resulted in the lowest LPO in the liver, but no significant difference was noticed when compared to the Mix diet on day 42 (p>0.05). Moreover, all of the tested extracts significantly reduced LPO in the gill on day 42. Pg0.2 and Mix diets were observed to be the lowest when compared to other diets (p<0.05).

Additionally, the activities of antioxidant enzymes in the gill and liver tissues of the control and challenged fish groups are depicted in Fig. 2 (A-B). Regardless of tissues, though temperature impacts significantly CAT in the liver and gill on day 14 (p<0.05), the temperature had no substantial effect on the activity of CAT in either tissue on day 42 (p>0.05).

The challenge temperature experiment revealed a significant impact of the plant extract-based diet on CAT in the liver. At the end of the study (day 42), the Pg0.2 supplemented group observed a significantly higher CAT in the liver, followed by the Mix group, compared to the Pa0.5 group and control group (p<0.05). Similar findings were obtained for CAT in the gill. This antioxidant enzyme was determined to have the highest value in the Pg02 diet when compared to the other diets.

4. DISCUSSION

Fish are ectothermic animals and temperature is the primary component that influences fish life, either directly or indirectly. Stress is the most common adverse consequence and is defined as any physical, biological, or chemical stimulus that

triggers physiological responses (Soltanian et al., 2014). Stress effects might relate to the water temperature variation triggered by a daily or seasonal shift (Ju *et al*., 2002). Temperature shock can harm fish by decreasing metabolic rates (Galloway and Kieffer, 2003), swimming behavior (Hocutt, 1973), affecting immunological systems (Hurst, 2007), lowering the capacity to acquire prey (Ward and Bonar, 2003), increasing susceptibility to diseases, and increasing mortality (Donaldson *et al*., 2008). A crucial aspect of the stress response is the redistribution of energy toward physiological processes that are not completely essential in support of processes that assist in restoring homeostasis (Fuzzen *et al*., 2011). Fish can adapt to stressors by rapidly increasing plasma glucose concentrations, which is not only the major energy source for fish but is also required by the brain and muscle tissues under stress conditions (Wendelaar-Bonga, 2011).

In this research, several haematological parameters reached high values at 31°C which differed significantly from the 27°C and 35°C groups. The results of some haematological parameters (RBCs, Hb, Hct) are in line with the research of aforementioned studies on *P. hypophthalmus* (Phuc, 2015; Shahjahane et al., 2018; Huong et al., 2020). An increase in haematological parameters such as RBCs, Hb, and Hct is a common response to anoxia or hypoxia (Hedayati and Tarkhani, 2014) and stress (Carvalho and Fernandes, 2006). For instance, Hedayati and Tarkhani (2014) revealed that in severe circumstances, RBCs, Hct, and Hb increased with the purpose to enhance blood oxygen-carrying capacity. Stress caused by temperature affected the physiological parameters of fish haematology including Hb, Hct, and cortisol levels (Roche and Bogé, 1996). Temperature can induce stress because it reduces the amount of dissolved oxygen in the water (Cech and Brauner, 2011). *P. hypophthalmus* can respond to heat stress by increasing the number of RBCs that increase Hb and Hct to ensure they meet higher oxygen requirements. At the level of 31°C, the value of some haematological parameters enhanced significantly compared to those in the 27°C group which was demonstrated similarly with the research of Huong et al. (2020) that some haematological parameters of this species increased at 30°C. In the thermal tolerance ranges, the temperature might act as a controlling factor via its effects on metabolism, then leading to the limits on maximum activity (Brett, 1964). *P. hypophthalmus* features not only an air bladder for air-breathing, but fish can also suffer to elevated temperature stress by altering their haemological characteristics to effectively meet high oxygen requirements (Roberts and Vidthayanon, 1991). In this research, there was no significant difference between the 31°C and 35°C groups after a 3-day high temperature. The RBCs, Hb, and Hct were lowest in the 35°C group which was in line with the study by Phuc (2015).

The efficiency of Pg0.2 inclusion in the diet was demonstrated in the results of Nhu et al. (2019); and Nhu et al., (2020) on the immunology of *P. hypophthalmus*. The administration of Pg0.2 in the present research improved the potential effect on the RBCs, Hb, and Hct in the experiment after 14-days of high-temperature exposure. The effect of Pg0.2 extract supplementation was in line with the study of Omitoyin et al., (2019) that after 84-day supplementation of *P. guajava* (0.75%) extract the highest values of Hb, and RBCs were recorded while control treatment had the least values for these parameters. In Nile tilapia research by Abdel-Tawwab and Hamed (2020), *P. guajava*-enriched (5 g/kg of feed) diet led to substantial increases in haematological parameters (RBCs, Hb, Hct) indicating a better haematological profile showing an enhancement of fish welfare. This might be explained by *P. guajava* comprises phenolic compounds that are attached to erythrocyte membranes, preventing cytotoxic damage. Some investigators also report that the antioxidants present in plant extracts might trigger erythropoiesis and the possible mechanism for erythropoiesis is a reduction in the oxidant-induced haemolysis rate due to the abundance of antioxidants in plant extracts (Maduinyi, 1983). The availability of active phenolic and antioxidant components in the leaves in both ethanol and aqueous extracts promotes its immunostimulatory ability on guava leaves (Laily et al., 2015) with the principal ingredients of the crude ethanol *P. guajava* leaf extract primarily being flavonoids and triterpene derivatives (Nhu et al., 2020).

The physiological functions involved in the fish's stress response were substantially affected by temperature. The release of stress hormones such as cortisol is the first step in the stress response in fish. A rise in plasma cortisol concentration in fish is frequently triggered by an increase in plasma glucose concentration. Biochemical markers such as plasma cortisol and glucose concentrations can be used to monitor stress in fish (Santos and Pacheco, 1996). This was seen in the non-statistically significant difference in glucose concentration

between treatment at 31°C and 35°C and the experiment with the comparatively clearest results was observed in the treatment of Pa0.2 extract. The result was in line with a study by Thinh et al. (2014), on the first days of the experiment, the glucose concentration elevated and was higher than those fish in the control group; nevertheless, following 14 days, this parameter gradually declined to normal values. Shahjahane et al. (2018) indicated that glucose concentration significantly increased at 36°C on day 3 and the current research revealed that *P. hypophthalmus* adapts better at 28°C and 32°C, but, a high temperature of 36°C is probably detrimental toward this fish species. Islam et al. (2019) indicated that glucose concentration was significantly increased at 36°C on day 7 in this species. Huong et al. (2020) found that fish were agitated at temperatures of 24°C and 36°C, as evidenced by an increase in hyperglycemia in plasma. Major responses to stressful conditions include increased gill blood circulation, locomotory activity, plasma glucose, gluconeogenesis, lower food intake, glycogen storage, and muscle proteins, growth, and reproduction, which are regulated by the brain and endocrine system (Wendelaar-Bonga, 2011). The blood glucose concentration tended to decrease on day 7 which is in line with the research of Phuc (2015) on *P. hypophthalmus*, after 4 days, glucose levels in the high-temperature conditions (34°C and 36°C) diminished. On day 7, physiological responses of altering haematological features caught up with oxygen consumption or energy utilization for swimming activity and were modulated with fish acclimation, resulting in no significant variations in plasma glucose concentrations.

The ability of plant extract on glucose reduction was demonstrated in research by Abdel-Tawwab and Hamed (2020), fish fed a *P. guajava* -enriched diet (5 g/kg) displayed significantly lower levels of glucose on *Oreochromis niloticus (L.)* levels as compared to the control group. Similarly, the lower serum glucose level observed in the treatment groups (*P. guajava* 1% and *Mangifera indica* 1%) compared with the control revealed that the *P. guajava* and *M. indica* leaves extracts possess a hypoglycaemic effect. This may be due to their capacity to mimic insulin, thus, improving the sensitivity of the cells to insulin and enhancing the peripheral utilization of glucose (Fawole, 2018). Temperature-stressed fish's gluconeogenesis reaction is thus an attempt to accommodate the energy requirements of the elevated temperature. (Barton, 2002). In another review article that there is a strong incentive for upcoming studies to examine the possible antistress utility of *P. guajava* leaves in experimental animals (Kamath et al., 2008). The elevated levels of adrenal cortical hormones during stress may be one of the explanations for the stress group's increased liver and adrenal gland weights. Pretreatment with *P. guajava* extract mitigate the stress-induced increase in liver and adrenal gland weight and prevented spleen atrophy. Because it comprises biologically active components such as flavonoids, quercetin, alkaloids, saponins, triterpenes, proteins, steroids, and fixed oils. Theses components suppress the clinical indications of the stress response (Lakshmi and Sudhakar, 2009).

In organisms subjected to a variety of adverse environmental conditions, notably temperature fluctuations, oxidative stress is a significant element of the stress response. Elevated temperatures have a profound impact, including protein structure abnormalities, enzyme denaturation, and cell membrane alterations. Consequently, fish experience stress from elevated temperatures in their neurological system, circulatory system, protein system, and enzymatic system (Chowdhury and Saikia, 2020). In the present investigation, liver tissues showed higher CAT activity, but the gill tissues recorded a higher LPO value. Nevertheless, water temperatures of 31°C and 35°C did not significantly differ in CAT activity and LPO value in the fish tissues during a long-term 42-day temperature exposure period ($p>0.05$) compared to a short-term 14-day period ($p<0.05$), which might be due to the metabolic process accelerated as the temperature changed, the fish did not acquire enough oxygen to supply the cells, leading to the release of release oxygen free radicals (ROS) during aerobic respiration. The ability of ectothermic animals' mitochondria for ADP re-phosphorylation and substrate oxidation is affected by temperature. Tropical fish can accelerate their metabolic rates by triggering their mitochondrial capability, which enhances at elevated temperatures, according to the Q_{10} correlation (Temperature coefficient - Q_{10} denotes the factor that determines how the reaction rate increases for every 10°C elevation in temperature.), and conversely, they decline at lower temperatures (Portner, 2011). Furthermore, according to Wendelaar-Bonga (2011), Stressors enhance the surface epithelia permeability to water and ions, including the gills, resulting in systemic hydromineral disturbances. The effects of

temperature on oxidative stress biomarkers have also been well described. Vinagre et al. (2012) discovered that *Dicentrarchus labrax* exposed to temperatures outside their thermal optimum exhibited the highest LPO and CAT activity. Briefly, at 28°C compared to 24°C, LPO and CAT elevated after 15 days, then declined to considerably lower levels after 30 days. A similar pattern of LPO and CAT activity was observed, CAT activities and LPO in the tissues assessed for the fish at 34°C were not significantly different from those of fish at 18°C after a long-term 60-day trial on *Garra rufa* (Uysal et al., 2019), which could be explained by the thermal adaptation of experimental fish during the experiment, in general, contributing to biological membrane remodeling and distinct metabolic alterations, and these physiological reactions might influence biological membrane sensitivity to LPO (Crockett, 2008). As a result, it is reasonable to assert that these fish have a relatively high thermal tolerance even at elevated water temperatures, such as 35°C.

Endogenous free radicals are present in the body as a consequence of normal metabolic processes such as cellular respiration, phagocytosis, and cell damage; thus, ROS are still generated under normal conditions (unaffected by external factors such as temperature, chemicals, etc.). The system produces antioxidant enzymes (SOD, CAT, etc) to protect cells from free radical damage. If an imbalance exists, however, an external antioxidant supplement is required (Valko et al., 2007). As a result, the utilization of plant extracts in fish feed is gaining momentum. According to the findings of this study, the Pg0.2 and Mix diets can effectively alter the activity of oxidative stress indicators. It was previously demonstrated that *P. guajava* possesses antioxidant properties since it prevents cell death, LPO, and hydrogen peroxide-induced ROS formation in zebrafish (Kim et al., 2016). Moreover, *P. guajava* diets (1 mg/g, 5 mg/g, and 10 mg/g) strengthened antioxidant enzymes such as SOD, CAT, and GPx in *O. mossambicus* compared to the control (Gobi et al., 2016). Additionally, *P. guajava* diets enhanced immunological response and antioxidant activity significantly increased in a dose-dependent manner on *Oreochromis niloticus* challenged with *Aeromonas hydrophila* (Omitoyin et al., 2019). Nantitanon et al. (2010) revealed that the active elements in *P. guajava* leaves comprise -sitosterol, flavonoids such as quercetin, leucocyanidin, avicularin, and guajaverin; and phenolic elements (including ellagic acid, gallic acid, morin, catechin, and quercetin) which absorb superoxide anions, so strengthening the nonspecific immunity of fish.

5. CONCLUSIONS

The Pg0.2 and Mix diets enhance the health of *P. hypophthalmus* through blood profile and oxidative stress biomarkers. The Pg0.2 or Mix inclusion is recommended to improve fish health and stress mitigation.

High temperature (35°C) had no significant impact on many haematological parameters (RBCs, Hct, Hb, glucose) until day 7 and oxidative stress indicators (LPO, CAT in gill and liver) until day 14. Afterward, the fish recovered and acclimated to the experimental conditions.

More upcoming studies are needed to investigate the effects of those plant extracts on fish health (especially histopathological and biomolecular aspects) as a crucial step toward their widespread application in farmed *P. hypophthamus*.

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REFERENCES

Abdel-Tawwab, M., & Hamed, H. S. (2020). Antagonistic effects of dietary guava (*Psidium guajava*) leaves extract on growth, hemato-biochemical, and immunity response of cypermethrin-intoxicated Nile tilapia, *Oreochromis niloticus*, fingerlings. *Aquaculture*, *529*, 735668.

Adeyemi, O. S., Akanji, M. A., & Ekanem, J. T. (2010). Anti-anaemic properties of the ethanolic extracts of *Psidium guajava* in *Trypanosoma brucei bruc*ei infected rats. *Research Journal of pharmacology*, *4*(3), 74-77.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and comparative biology*, *42*(3), 517-525.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Board of Canada*, *21*(5), 1183-1226.

Carvalho, C. S., & Fernandes, M. N. (2006). Effect of temperature on copper toxicity and hematological responses in the neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture*, *251*(1), 109-117.

Cech, J. J., Brauner, C. J., 2011. Respiration: An Introduction. In: Farrell, A.p. (Ed.), *Encyclopedia of fish physiology: From genome to environment*. Elsevier, pp. 791-795.

Cheng, F. C., Shen, S. C., & Wu, J. S. B. (2009). Effect of guava (*Psidium guajava* L.) leaf extract on glucose uptake in rat hepatocytes. *Journal of food science*, *74*(5), H132-H138.

Chowdhury, S., & Saikia, S. K. (2020). Oxidative stress in fish: a review. *Journal of Scientific Research*, *12*(1), 145-160.

Crockett, E. L. (2008). The cold but not hard fats in ectotherms: consequences of lipid restructuring on susceptibility of biological membranes to peroxidation, a review. *Journal of Comparative Physiology B*, *178*(7), 795-809.

Dao, L. A. N., Phu, T. M., Douny, C., Quetin-Leclercq, J., Hue, B. T. B., Bach, L. T., ... & Scippo, M. L. (2020). Screening and comparative study of in vitro antioxidant and antimicrobial activities of ethanolic extracts of selected Vietnamese plants. *International Journal of Food Properties*, *23*(1), 481-496.

Donaldson, M. R., Cooke, S. J., Patterson, D. A., & Macdonald, J. S. (2008). Cold shock and fish. *Journal of Fish Biology*, *73*(7), 1491-1530.

Fatima, M., Ahmad, I., Sayeed, I., Athar, M., & Raisuddin, S. (2000). Pollutant-induced over-activation of phagocytes is concomitantly associated with peroxidative damage in fish tissues. *Aquatic Toxicology*, *49*(4), 243-250.

Fawole, F. J., Sahu, N. P., Nazeemashahul, S., & Adeoye, A. A. (2018). Effect of *Psidium guajava* and *Mangifera indica* leaves extracts on growth, antioxidant and metabolic enzymes activities of *Labeo rohita* fingerlings. *Journal of the Association of Nigerian Fisheries Scientists*, *1*, 40-49.

Ferdous, J., Hossain, M. M. M., Jaman, M. H. U., Rupom, A. H., Tonny, N. I., & Jaman, A. (2017). *Psidium guajava* leaf extracts fed to mono-sex Nile tilapia *Oreochromis niloticus* enhance immune response against Pseudomonas fluorescens. *European Journal of Clinical and Biomedical Sciences*, *3*(1), 34-42.

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. In *Fish physiology* (Vol. 6, pp. 1-98). Academic press.

Fuzzen, M. L. M., Bernier, N. J., Kraak, G. V. D., 2011. Stress and Reproduction. In: Norris, D. O., Lopez, K. H. (Eds.), *Hormones and Reproduction of Vertebrates*. Academic Press, pp. 103-118

Galloway, B. J., & Kieffer, J. D. (2003). The effects of an acute temperature change on the metabolic recovery from exhaustive exercise in juvenile Atlantic salmon (*Salmo salar*). *Physiological and Biochemical Zoology*, *76*(5), 652-662.

Gobi, N., Ramya, C., Vaseeharan, B., Malaikozhundan, B., Vijayakumar, S., Murugan, K., & Benelli, G. (2016). *Oreochromis mossambicus* diet supplementation with *Psidium guajava* leaf extracts enhance growth, immune, antioxidant response and resistance to *Aeromonas hydrophila*. *Fish & Shellfish Immunology*, 58, 572-583.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, *196*(2-3), 143-151.

Gupta, V. K., & Sharma, S. K. (2006). Plants as natural antioxidants. *Natural Product Radiance 5*(4), 326-334.

Halliwell, B., & Gutteridge, J. M. (2015). *Free radicals in biology and medicine*. Oxford university press, USA.

Hedayati, A., & Tarkhani, R. (2014). Hematological and gill histopathological changes in iridescent shark, *Pangasius hypophthalmus* (Sauvage, 1878) exposed to sublethal diazinon and deltamethrin concentrations. *Fish Physiology and Biochemistry*, *40*(3), 715-720.

Hocutt, C. H. (1973). Swimming performance of three warm water fishes exposed to a rapid temperature change. *Chesapeake Science*, *14*(1), 11-16.

Huggett, A. S. G. (1957). Enyzmic determination of blood glucose. *Biochem. J.*, *66*, 12.

Huong, D. T. T, Ha, N. T. K., Ngoc, N. M., Em, N. T, Toyoji Kaneko, Phuong, N. T. (2020). Effects of temperature on physiology, growth performance, and digestive enzyme activity of striped catfish (*Pangasianodon hypophthalmus*) from fry to small fingerling stage. *Can Tho University Journal of Science*, *56*(1-11) (In Vietnamese).

Hurst, T. P. (2007). Causes and consequences of winter mortality in fishes. *Journal of Fish Biology*, *71*(2), 315-345.

Imsland, A. K., Björnsson, B. T., Gunnarsson, S., Foss, A., & Stefansson, S. O. (2007). Temperature and salinity effects on plasma insulin-like growth factor-I concentrations and growth in juvenile turbot

(*Scophthalmus maximus*). *Aquaculture*, *271*(1-4), 546-552.

IPCC (2013). *Climate change 2013: The Physical Science Basis.* Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate change, Working Group I contribution to the IPCC Fifth Assessment Report (AR5). Cambridge Univ Press, New York, 1535.

Islam, M. A., Uddin, M. H., Uddin, M. J., & Shahjahan, M. (2019). Temperature changes influenced the growth performance and physiological functions of Thai pangas *Pangasianodon hypophthalmus*. *Aquaculture Reports*, *13*, 100179.

Ju, Z., Dunham, R. A., & Liu, Z. (2002). Differential gene expression in the brain of channel catfish (*Ictalurus punctatus*) in response to cold acclimation. *Molecular Genetics and Genomics*, *268*(1), 87-95.

Kamath, J. V., Rahul, N., Kumar, C. A., & Lakshmi, S. M. (2008). *Psidium guajava* L: A review. *International Journal of Green Pharmacy (IJGP)*, *2*(1).

Kemp, J. O. G. (2009). Effects of temperature and salinity on resting metabolism in two South African rock pool fish: the resident gobiid *Caffrogobius caffer* and the transient sparid *Diplodus sargus* capensis. *African Zoology*, *44*(2), 151-158.

Kim, S. Y., Kim, E. A., Kim, Y. S., Yu, S. K., Choi, C., Lee, J. S., ... & Jeon, Y. J. (2016). Protective effects of polysaccharides from *Psidium guajava* leaves against oxidative stresses. *International Journal of Biological Macromolecules*, *91*, 804-811.

Laily, N., Kusumaningtyas, R. W., Sukarti, I., & Rini, M. R. D. K. (2015). The potency of guava *Psidium guajava* (L.) leaves as a functional immunostimulatory ingredient. *Procedia Chemistry*, *14*, 301-307.

Lakshmi, B. V. S., & Sudhakar, M. (2009). Screening of *Psidium guajava* leaf extracts for antistress activity in different experimental animal models. *Pharmacognosy research*, *1*(6).

Larsen, H. N., & Snieszko, S. F. (1961). Modification of the microhematocrit technique with trout blood. *Transactions of the American Fisheries Society*, *90*(2), 139-142.

Law of animal health, 2015. Article 21 - Vietnam national assembly Passes animal health law – Date: 10/7/2015, Report number: VM5068.

Lesser, M. P. (2006). Oxidative stress in marine environments: biochemistry and physiological ecology. *Annual Review of Physiology*, *68*, 253-278.

Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, *101*(1), 13-30.

MARD_Ministry of Agriculture and Rural Development (2022). Tra fish exports towards the target of over 1.6 billion USD in 2022. https://www.mard.gov.vn/en/Pages/tra-fish-exports-towards-the-target-of-over-1-6-billion-usd-in-2022.aspx. Accessed on 8/5/2022.

MONRE (Ministry of Natural Resources and Environment) (2009). *Scenarios for climate change, sea level rise for Vietnam*. 34 pages.

Maduinyi, I. (1983). *Biochemical and pharmiocol studies of the active principles of the seeds of Garcinia kola* (Doctoral dissertation, University of Nigeria Nsukka, Nigeria).

Nantitanon, W., Yotsawimonwat, S., & Okonogi, S. (2010). Factors influencing antioxidant activities and total phenolic content of guava leaf extract. *LWT-Food Science and Technology*, *43*(7), 1095-1103.

Natt, M. P., & Herrick, C. A. (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poultry Science*, *31*(4), 735-738.

Nhu, T. Q., Bich Hang, B. T., Cornet, V., Oger, M., Bach, L. T., Anh Dao, N. L., ... & Kestemont, P. (2020). Single or combined dietary supply of *Psidium guajava* and *Phyllanthus amarus* extracts differentially modulate immune responses and liver proteome in striped catfish (*Pangasianodon hyphophthalmus*). *Frontiers in Immunology*, *11*, 797.

Nhu, T. Q., Hang, B. T. B., Vinikas, A., Hue, B. T. B., Quetin-Leclercq, J., Scippo, M. L., ... & Kestemont, P. (2019). Screening of immuno-modulatory potential of different herbal plant extracts using striped catfish (*Pangasianodon hypophthalmus*) leukocyte-based in vitro tests. *Fish & Shellfish Immunology*, *93*, 296-307.

Oh, W. K., Lee, C. H., Lee, M. S., Bae, E. Y., Sohn, C. B., Oh, H., ... & Ahn, J. S. (2005). Antidiabetic effects of extracts from *Psidium guajava*. *Journal of Ethnopharmacology*, *96*(3), 411-415.

Omitoyin, B. O., Ajani, E. K., Orisasona, O., Bassey, H. E., Kareem, K. O., & Osho, F. E. (2019). Effect of guava *Psidium guajava* (L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Aquaculture Research*, *50*(7), 1851-1861.

Pachanawan, A., Phumkhachorn, P., & Rattanachaikunsopon, P. (2008). Potential of *Psidium guajava* supplemented fish diets in controlling *Aeromonas hydrophila* infection in tilapia (*Oreochromis niloticus*). *Journal of Bioscience and Bioengineering*, *106*(5), 419-424.

Phuc, N. T. H. (2015). *Effects of temperature and salinity on growth performance in cultured tra catfish (Pangasianodon hypophthalmus) in Vietnam* (Doctoral dissertation, Queensland University of Technology), 159 pages.

Portner, H. O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., ... & Zakhartsev, M. (2001). Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). *Continental Shelf Research*, *21*(18-19), 1975-1997.

Portner, H. O. (2011). Cellular Energy Utilization: Environmental Influences on Metabolism, in: Farrell, A.P. (Ed.), *Encyclopedia of Fish Physiology: From Genome To Environment*. Academic Press, pp. 1645-1651.

Roberts, T. R., & Vidthayanon, C. (1991). Systematic revision of the Asian catfish family Pangasiidae, with biological observations and descriptions of three new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 97-143.

Roche, H., & Bogé, G. (1996). Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Marine Environmental Research*, *41*(1), 27-43.

Santos, M. A., & Pacheco, M. (1996). *Anguilla anguilla*L. Stress Biomarkers Recovery in Clean Water and Secondary-Treated Pulp Mill Effluent. *Ecotoxicology and Environmental Safety*, *35*(1), 96-100.

Semren, T. Ž., Žunec, S., & Pizent, A. (2018). Oxidative stress in triazine pesticide toxicity: a review of the main biomarker findings. *Archives of Industrial Hygiene and Toxicology*, *69*(2), 109-125.

Shahjahan, M., Uddin, M., Bain, V., & Haque, M. (2018). Increased water temperature altered hemato-biochemical parameters and structure of peripheral erythrocytes in striped catfish *Pangasianodon hypophthalmus*. *Fish Physiology and Biochemistry*, *44*(5), 1309-1318.

Shakeera, B. M., Sujatha, K., Sridharan, G., & Manikandan, R. (2013). Antihyperglycemic and antihyperlipidemic potentials of *Psidium guajava* in alloxan-induced diabetic rats. *Asian Journal of Pharmaceutical and Clinical Research*, *6*(2), 88-89.

Shen, S. C., Cheng, F. C., & Wu, N. J. (2008). Effect of guava (*Psidium guajava* Linn.) leaf soluble solids on glucose metabolism in type 2 diabetic rats. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *22*(11), 1458-1464.

Snellgrove, D. L., & Alexander, L. G. (2011). Haematology and plasma chemistry of the red top ice blue mbuna cichlid (*Metriaclima greshakei*). *British Journal of Nutrition*, *106*(S1), S154-S157.

Soltanian, S., Adloo, M. N., Hafeziyeh, M., & Ghadimi, N. (2014). Effect of β-Glucan on cold-stress resistance of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878). *Veterinarni Medicina*, *59*(9), 440-446.

Thinh, P. V., Phuong, N. T., Huong, D. T. T., & Phuc, N. T. H. (2014). The effects of temperature on physiological parameters and growth rate of catfish (*Pangasianodon hypophthalmus*). *Can Tho University Journal of Science*, *1*, 292-301.

Tobin, D., & Wright, P. J. (2011). Temperature effects on female maturation in a temperate marine fish. *Journal of Experimental Marine Biology and Ecology*, *403*(1-2), 9-13.

Uysal, K., Çolak, Y., & Kavasoğlu, M. (2019). Effects of Water Temperature on Catalase Activity and Malondialdehyde Level in Muscle, Mouth, and Intestine Tissues of Doctor Fish (*Garra rufa*). *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, *23*(1), 191-194.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international Journal of Biochemistry & Cell Biology*, *39*(1), 44-84.

Van Weerd, J. H., & Komen, J. (1998). The effects of chronic stress on growth in fish: a critical appraisal. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *120*(1), 107-112.

Vinagre, C., Madeira, D., Narciso, L., Cabral, H. N., & Diniz, M. (2012). Effect of temperature on oxidative stress in fish: lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. *Ecological Indicators*, *23*, 274-279.

Ward, D. L., & Bonar, S. A. (2003). Effects of cold water on susceptibility of age-0 flannelmouth sucker to predation by rainbow trout. *The Southwestern Naturalist*, 43-46.

Wendelaar-Bonga, S. E. (2011). Hormonal response to stress. In: Farrel, A.P. (Ed.), *Encyclopedia of Fish: Fish Physiology From Genome to Environment*. Academic Press, pp. 1515-1523.

Zijlstra, W. G., Buursma, A., & Zwart, A. (1983). Molar absorptivities of human hemoglobin in the visible spectral range. *Journal of Applied Physiology*, *54*(5), 1287-1291.