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Research article

# Effects of dietary supplementation of spray dried hog plum (*Spondias pinnata* (L.f.) Kurz) fruit powder on growth, digestive enzyme activity, and skin mucus immune parameters of climbing perch (*Anabas testudineus* (Bloch, 1972))

**Wilawan Promprom[1,2], Wannachai Chatan[1,2], Somsak Khambaione[3], Kajita Somnate[4] and Phukphon Munglue[2,5,*]**

*[1]Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham 44150, Thailand*
*[2]Plant and Innovation Research Unit, Mahasarakham University, Maha Sarakham 44150, Thailand*
*[3]Pakse Teachers Training College, Km 3, 13 South Road, Pakse 031212262, Laos*
*[4]Program of Applied Statistics, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand*
*[5]Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand*

## Abstract

The effects of dietary supplementation of spray-dried hog plum (*Spondias pinnata* (L.f.) Kurz) fruit powder (SPP) on growth, digestive enzyme activity, and skin mucus immune parameters of climbing perch (*Anabas testudineus* (Bloch, 1972)) were examined. The fish (N = 330; 3.58±0.12 g) were assigned to five groups in triplicates. They were fed different SPP levels at 0, 5, 10, 15, and 20 g/kg diet for eight weeks. The phytochemical analysis of SPP revealed the presence of flavonoids, terpenoids, phenolic compounds, tannins, steroids, and glycosides. The fruit powder exhibited strong DPPH-radical scavenging activity. The SPP containing diet significantly improved growth parameters ($P < 0.05$) without a negative effect on the survival rate ($P > 0.05$). Intestinosomatic and hepatosomatic indices significantly declined in the fish fed SPP added diets ($P < 0.05$). There were significant elevations in digestive enzyme activities, amylase, protease, lipase, trypsin, pepsin, and esterase, in SPP groups ($P < 0.05$). Additionally, fish fed SPP containing diets showed a significant enhancement of total protein, immunoglobulin M, lysozyme, antiprotease, alkaline phosphatase, and esterase in the skin mucus ($P < 0.05$). Significantly higher levels of superoxide dismutase, catalase, and total antioxidant capacity were detected in fish fed SPP containing diets compared to the control ($P < 0.05$). These findings suggest that dietary SPP supplementation can effectively enhance fish growth, digestive enzyme activities, and mucosal immune parameters in the climbing perch. The optimum level of SPP observed in this present study was 13.00 – 15.88 g/kg diet.

**Keywords:** Climbing perch, Digestive enzyme activity, Growth, Hog plum, Immunostimulant

***Corresponding author:** Phukphon Munglue, Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand. E-mail: phukphon.m@ubru.ac.th.

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# INTRODUCTION

Climbing perch (*Anabas testudineus* (Bloch, 1972)) is one of the most important economic fish species, typically occurring in rivers, canals, ditches, swamps, and rice fields. Its meat contains various chemical components such as proteins, unsaturated fatty acids, and vitamins (Bureau of Nutrition, 2018). Iron and copper are the primary nutrient minerals commonly found in its flesh and are essential for biological electron transport and oxygen transportation in animals (Luo et al., 2022). However, the population of this species in nature has declined because of climate change, habitat loss and degradation, and overexploitation. Climbing perch has been introduced and cultivated in extensive and intensive rearing conditions in Bangladesh, India, Thailand, Malaysia, and Vietnam to increase fish production (Hossain et al., 2012). The significant limitations of intensive production are environmental conditions, water quality, feeding requirements, animal health implications, and disease outbreaks (Hoseinifar et al., 2020). The use of chemicals and therapeutics in aquaculture production is an attempt to enhance growth rate, prevent diseases, and treat bacterial and fungal infections, which can produce serious environmental, animal, and public health hazards because of the development of antibiotic-resistant bacteria (Ahmed et al., 2020). Therefore, applying phytochemicals as growth-promoting, antipathogenic, and immunostimulants in fish farming to reduce environmental, animal, and human health risks is now required (Jiang et al., 2016; Khunchalee and Munglue, 2020; Huang et al., 2022).

*Spondias pinnata* (L.f.) Kurz (Anacardiaceae), known as hog plum or wild mango, is a perennial evergreen tree predominantly found in tropical and subtropical regions throughout the globe. This plant's tender tips and fruit are commonly consumed as a vegetable. Powdered and dried leaves have been used to treat gastroenteritis (Swathi and Lakshman, 2022). The root has been used to regulate menstruation (Laksemi, 2019). The fruits are applied to alleviate laryngitis and rheumatism (Sameh et al., 2018). As noted, hog plums have many biological activities, such as anticarcinogenic, antioxidant, antimicrobial, anti-inflammatory, antihyperglycemic, and anti-helminthic (Sameh et al., 2018; Swathi and Lakshman, 2022). It is thought that the potential health benefits of hog plum may be due to several active components, including alkaloids, tannins, saponins, glycosides, terpenoids, flavonoids, polyphenols, essential oils, amino acids, carbohydrates, vitamins, and minerals (Laksemi, 2019). Recently, a study on nutrient composition per 100-gram edible portion of hog plum fruit showed the presence of 0.70 g protein, 0.20 g fat, 161 mg calcium, 36 mg phosphorus, 1.30 mg iron, 27 µg vitamin A, 1.40 mg niacin, and 9 mg vitamin C (Bureau of Nutrition, 2018).

Spray drying has long been used to produce solid or semi-solid powders from fluid samples in the pharmaceutical, food, and chemical industries (Singh et al., 2019). Some of the notable benefits of this method are to improve the shelf life, decrease weight or volume, reserve materials for year-around availability, and reduce the degradation of phytochemical compounds (Chang et al., 2022). Thus, spray drying methods could be useful in the near future for preparing some feed ingredients, natural additives, and drugs in aquaculture industries.

Final weight, specific growth rate, and weight gain are growth parameters commonly employed to monitor the direct effects of feeding regimes or tested diets in experimental fish. Observations of digestive enzyme activities,

such as amylase, protease, lipase, and trypsin, can serve as valuable tools for estimating digestive and metabolic capacity, as well as assessing the response of these enzymes to diet formulations. Superoxide dismutase and catalase are antioxidant enzymes detectable in the skin mucus of fish, which play a crucial role in preventing oxidative stress resulting from the overproduction of free radicals (Habotta et al., 2022). Skin mucus comprises a non-specific immune system with a wide array of vital immunological components, including total protein, immunoglobulin M, alkaline phosphatase, lysozyme, and antiprotease. Fish skin mucus acts as both a physical and chemical barrier against invading pathogens and toxins. It is well-documented that herbal plants contain numerous active ingredients with growth-promoting, antioxidant, and immunomodulatory properties (Awad and Awaad, 2017; Khunchalee and Munglue, 2020; Adel et al., 2021). Several studies have demonstrated that incorporating medicinal plants or their active components into diets can enhance various aspects of fish, including growth performance parameters, digestive enzyme activities, and immune responses (Habotta et al., 2022; Vijayaram et al., 2022). Adel et al. (2021) reported that Siberian sturgeon (*Acipenser baerii*) fed diets containing lemon verbena (*Aloysia citrodora*) at 0, 5, 10, and 20 g/kg for 8 weeks exhibited enhancements in growth performance, intestinal enzyme activities, and skin mucus immune responses compared to the control group. Dietary supplementation with *Pistacia vera* hull-derived polysaccharides at different levels (0, 2.5, 5, and 10 g/kg diet) for 60 days resulted in increased growth performance, digestive enzyme activity, antioxidative capacity, and immune response in Nile tilapia (*Oreochromis niloticus*) (Mohammadi et al., 2020).

To our knowledge, the effect of dietary supplementation of spray-dried hog plum fruit powder (SPP) on fish growth and health has yet to be tested. As there is an urgent need to find novel drugs to enhance the growth performance of fish, and several compounds are required (Awad and Awaad, 2017; Vijayaram et al., 2022), the aim of this present study was, therefore, to investigate the effects of dietary supplementation of SPP on growth performance, digestive enzyme activity, and skin mucus immune parameters of climbing perch.

## MATERIALS AND METHODS

### Ethics approval

Climbing perch fingerlings used in this study were obtained from Ubon Ratchathani Fisheries Cooperative, Ubon Ratchathani, Thailand. The animals were maintained under the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Thailand. The experimental protocol No. AN64009 was approved by the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand.

### Plant preparation

Fresh ripe hog plum fruits were collected in the rainy season from Kham Riang Subdistrict, Kantharawichai District, Maha Sarakham, Thailand and identified by a botanist at the Department of Biology, Faculty of Science, Mahasarakham University. The plant specimens were kept at the Department of Biology, Faculty of Science, Mahasarakham University. The fruit samples were cleaned to remove some impurities using tap water and dried using a hot air oven at 60°C for three days.

## Hog plum fruit powder preparation

One hundred grams of dried fruits were boiled in 1 L of water (100°C) for 15-20 minutes. The mixture was then chilled, filtered using a cotton filter, and kept in a refrigerator for spray drying. The filtrate was mixed with maltodextrin solution at 10% (% w/w) to give a homogenous mixture. The liquid was fed into a Büchi mini spray dryer (Büchi Labortechnik AG, B-290, Switzerland) to produce a hog plum powder sample. The powder was placed in an amber glass bottle and stored at -20°C until use. The process yield (%) of the hog plum power was calculated by using the following equation (Chang et al., 2022):

Process yield (%) = [Dried hog plum power (g)/(sieved hog plum juice (g) + maltodextrin (g)] × 100

## Qualitative phytochemical analysis

Alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, steroids, saponins, coumarins, anthraquinones, and glycosides in SPP were assessed according to the report of Tacouri et al. (2013).

## Quantitative phytochemical analysis

The total phenolic content of SPP was determined using the Folin-Ciocalteu method as described by Pintapagung and Asawapattanakul (2020). The absorbance was measured at 765 nm using a spectrophotometer (Eppendorf, Germany) against a reagent blank, and a calibration curve with gallic acid was prepared. The results were reported as milligrams of gallic acid equivalent (mg GAE/g of extract).

Total flavonoid content was estimated using the procedure outlined by Men et al. (2022). The absorbance of the reaction mixture was measured at 510 nm, and a calibration curve with quercetin was constructed. The total flavonoid content was expressed as milligrams of quercetin equivalent (mg QE/g of extract).

## DPPH radical-scavenging activity

The DPPH radical-scavenging activity of SPP was assessed using the method outlined by Pintapagung and Asawapattanakul (2020), with slight modifications. Various concentrations of SPP were mixed with an equal volume of DPPH methanol solution. After incubating for 30 minutes, the samples were measured at 515 nm against methanol as a blank using a UV-visible spectrophotometer (UV-1700 Shimadzu, Japan). The DPPH radical-scavenging activity of SPP was calculated using the following equation:

DPPH radical-scavenging activity (%) = [(Control absorbance – Sample absorbance)/ Control absorbance] × 100

$IC_{50}$ values were also determined and expressed as the concentration required for a 50% inhibition of the DPPH radical. Ascorbic acid served as the reference standard.

## Diet preparation

The spray-dried hog plum powder at 0, 5, 10, 15, and 20 g was incorporated into one kg of the basal fish diet containing 42% crude protein, 4% crude lipid, 3% fiber, and 10% moisture. The fish diets were ground, mixed with spray-dried hog plum powder, producing pellets 2.0 mm in size. The experimental diets were dried in an oven at 40°C and kept in a sealed plastic bag in a refrigerator at 4°C until use. The levels used in this research were chosen based on information reported by Pratheepa and Sukumaran (2014) with a minor modification.

## Experimental protocols

Climbing perch fingerlings were acclimatized for two weeks in Ubon Ratchathani Rajabhat University Fishery Farm in the 1000 L tank with consistent aeration. External appearance, general health and well-being, and disease symptoms were observed daily. Fish were fed a commercial diet containing 42% crude protein, 4% crude lipid, 3% fiber, and 10% moisture. After acclimatization, 330 fingerlings (3.58±0.12 g) were assigned to five treatments with three replicates. Fish were cultured in circular cement concrete tanks equipped with constant aeration at a stock density of 22 fish per tank for eight weeks. 50% of the water in each tank was changed with fresh water daily. The water quality parameters, including pH (7.21-7.82), temperature (25.3-28.2°C), total hardness (50-100 mg/L), alkalinity (25-100 mg/L), biochemical oxygen demand (BOD) (3-20 mg/L), ammonia (< 0.025 mg/L), nitrite (< 0.02 mg/L), nitrate (0.1-4.5 mg/L), and dissolved oxygen (5.7-6.5 mg/L), were measured two times a day at 8.00 a.m. and 2.00 p.m. to maintain at the optimum levels for the fish culture. If dead fish were present, they were removed, and the data were recorded.

## Growth performance

At the end of the experimental period, fish in each tank were starved for 24 h, counted, and weighed. Growth performance parameters were calculated using the following formulae.

Weight gain (WG; g) = final body weight (g) – initial body weight (g);

Specific growth rate (SGR; %/day) = 100 × [Ln final body weight (g) – Ln initial body weight (g)]/experimental period (day);

Average daily gain (ADG; g/day) = final body weight (g)/experimental period (day);

Feed intake (FI; g feed/fish) = dry feed fed (g)/number of fish;

Feed conversion ratio (FCR) = dry feed fed (g)/weight gain (g);

Survival rate (SR; %) = 100 × [final number of fish/initial number of fish].

## Digestive enzyme study

At the end of the experiment, fish were starved for 24 h before sample collection. Four fish from each tank (12 per treatment) were randomly sampled, weighed, and euthanized with actual dose of clove oil. Fish were transferred to a dissection tray and checked carefully for their consciousness. The abdomen was opened. The intestine and liver were collected, cleared from the adherent tissues, and weighed. The intestinal tissue samples were homogenized in 50 mM Tris-HCl buffer (pH 7.5) containing 200 mM NaCl (1:5 weight: volume). The homogenate was placed in an Eppendorf tube and centrifuged at 10000 ×*g* for 20 minutes at 4°C. The supernatants were collected and stored at -80°C for enzyme activity analysis (Raissy et al., 2022).

The amylase activity was evaluated using the 3,5-dinitro salicylic acid (DNS) assay (Thongprajukaew et al., 2010). Non-specific protease activity was determined using 1% casein as a substrate (Cupp-Enyard, 2008). Lipase activity was estimated using 0.01 M p-nitrophenyl palmitate (pNNP) as a substrate, as described by Thongprajukaew et al. (2010). Trypsin activity was detected using 50 mM N-α-benzoyl-DL-arginine-p-nitroanalidine (BApNA) as a substrate

(Natalia et al., 2004). Pepsin assay used 2% casein as a substrate (Hahor et al., 2016). Esterase activity was evaluated using 2.5 mM 4-Nitrophenyl acetate as a substrate (Pavarina et al., 2021). The total protein content in the supernatant was determined according to the Lowry method, using bovine serum albumin as the standard (Lowry et al., 1951). Specific enzyme activities were expressed in U per mg protein in the samples and calculated as follows:

$$\text{Specific enzyme activities (U/mg total protein)} = \frac{\Delta Abs \times Vtotal}{\varepsilon \times Vsample \times t} \times \frac{mL}{\text{mg total protein}}$$

Where ΔAbs is an increase of the absorbance at a determined wavelength; Ɛ is the molar extinction coefficient; Vtotal is the total volume of the reaction; Vsample is the volume of the enzyme extract; t is the reaction time.

## Skin mucus collection

Four fish from each tank (12 per treatment) were collected and euthanized with actual dose of clove oil. Fish were then transferred into a polyethylene bag containing 10 mL of 50 mM NaCl and kept for two minutes. The skin mucus was put in 15 mL sterile tubes and centrifuged at 5000 x g at 4°C for 10 minutes. The supernatants were harvested and kept in an Eppendorf tube at -20°C until further analysis.

## Skin mucus immune determination

The total protein level in the mucus was estimated using bovine serum albumin as the standard, based on the method of Lowry et al. (1951). Immunoglobulin M (IgM) value was obtained as reported by Raissy et al. (2022). Mucosal lysozyme activity was examined by a turbidimetric method based on the lysis of *Micrococcus lysodeikticus*, a gram-positive bacterium (Sigma-Aldrich, USA). The activity of alkaline phosphatase (ALP) was determined by the IFCC method, using 4-nitrophenyl phosphate as a substrate (Tietz et al., 1983). Antiprotease activity was determined based on Zuo and Woo (1997), using trypsin (bovine pancreas type I, Sigma-Aldrich) as a substrate. Esterase activity was estimated using 4-nitrophenyl acetate as a substrate (Pavarina et al., 2021). Myeloperoxidase (MPO) content was measured according to Quade and Roth (1997).

## Skin mucus antioxidant activities

Superoxide dismutase (SOD) activity was measured based on nitro blue tetrazolium reduction by $O_2^{-}$ radical (Mohammadi et al., 2020). The rate of disappearance of hydrogen peroxide produced by the mucus sample was used to determine the catalase (CAT) activity, as previously suggested by Mohammadi et al. (2020). The total antioxidant capacity (TAC) in the fish mucus was investigated by the Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996).

## Statistical analysis

In this research, a Completely Randomized Design (CRD) was used. The results are expressed as mean ± standard error of the mean (SEM). All data were submitted to test the normal distribution and variance homogeneity using the Kolmogorov-Sminov and Levene tests. If the data showed non-normal distribution, the arcsin square-root transformation was used. The data were

then subjected to one-way ANOVA followed by the Duncan test. Significant differences were obtained when $P < 0.05$. The suitable level of SPP was calculated using second-order polynomial regression.

# RESULTS

## Phytochemical analysis and DPPH radical-scavenging activity

The process yield of SPP obtained in this research was 1.6%. Phytochemical analysis of SPP showed positive results for flavonoids, terpenoids, phenolic compounds, tannins, steroids, and glycosides. However, alkaloids, saponins, coumarins, and anthraquinones were not detected in SPP, as shown in Table 1. The total phenolic and total flavonoid contents of SPP were 204.70±2.06 mg GAE/g of extract and 172.53±1.65 mg QE/g of extract, respectively (see Table 2). The percentage of free radical scavenging activity exhibited by SPP was 51.36%. The DPPH inhibitory concentration ($IC_{50}$) of SPP is presented in Table 2. The results showed that the $IC_{50}$ value of SPP (224.64±1.81 µg/mL) was significantly lower than that of ascorbic acid (34.66±0.65 µg/mL) ($P < 0.05$).

**Table 1** Phytochemical screening of SPP

| Phytochemicals | Test results |
|---|---|
| Alkaloids | - |
| Flavonoids | + |
| Terpenoids | + |
| Phenolic compounds | + |
| Tannins | + |
| Steroids | + |
| Saponins | - |
| Coumarins | - |
| Glycosides | + |
| Anthraquinones | - |

The results obtained were performed in triplicate. + = present, - = absent.

**Table 2** Total phenolic content, total flavonoid content, and DPPH radical-scavenging activity of SPP

| Samples | Total phenolic content (mg GAE/g of extract) | Total flavonoid contents (mg QE/g of extract) | DPPH radical-scavenging activity (%) | DPPH radical scavenging activity ($IC_{50}$ µg/ml) |
|---|---|---|---|---|
| SPP | 204.70±2.06 | 172.53±1.65 | 51.36 | 224.64±1.81$^{b}$ |
| Ascorbic acid | - | - | - | 34.66±0.65$^{a}$ |

Results are presented as mean ± SEM (n = 3). Superscripts in the same column indicate significant differences among the treatments ($P < 0.05$). GAE = gallic acid equivalent, QE = quercetin equivalent, $IC_{50}$ = the concentration required for a 50% inhibition of the DPPH radical.

**Growth performances**

The effects of dietary SPP on the growth of climbing perch are summarized in Table 3. The final weight (FW), WG, SGR, ADG, and FI of fish fed with SPP containing diets were found to be higher than those of fish fed the control diet ($P < 0.05$). In addition, fish fed with the SPP diets showed a significant decrease in FCR levels compared with the control ($P < 0.05$). The results also exhibited no significant difference ($P > 0.05$) between the experimental fish and the control fish in the values of SR. Based on the second-order polynomial regression analysis on FW ($y = -0.070x^2 + 2.210x + 20.967$, $R^2 = 0.252$, $P = 0.000$), WG ($y = -0.069x^2 + 2.191x + 17.451$, $R^2 = 0.250$, $P = 0.000$), SGR ($y = -0.004x^2 + 0.125x + 3.200$, $R^2 = 0.276$, $P = 0.000$), ADG ($y = -0.002x^2 + 0.052x + 0.296$, $R^2 = 0.315$, $P = 0.000$), and FCR ($y = 0.005x^2 – 0.153x + 2.398$, $R^2 = 0.431$, $P = 0.000$), the suitable level of SPP was 13.00 – 15.88 g SPP/kg diet as presented in Figure 1. As demonstrated in Figure 2, the relative organ weight of the intestine and liver in fish fed with the SPP diets was significantly decreased compared to the control fish ($P < 0.05$).

**Table 3** Growth performance of climbing perch fed diets containing various levels of SPP for 8 weeks

| Parameters | SPP levels (g/kg diet) | | | | |
|---|---|---|---|---|---|
| | Control | 5 | 10 | 15 | 20 |
| IW (g) | 3.55±0.12 | 3.50±0.11 | 3.66±0.14 | 3.66±0.10 | 3.55±0.12 |
| FW (g) | 20.77±2.77$^{c}$ | 31.22±2.30$^{b}$ | 34.33±2.22$^{ab}$ | 39.72±3.73$^{a}$ | 36.77±3.29$^{ab}$ |
| WG (g) | 17.22±1.73$^{c}$ | 27.72±2.32$^{b}$ | 30.66±2.23$^{ab}$ | 36.05±3.71$^{a}$ | 33.22±3.30$^{ab}$ |
| SGR (%/day) | 3.15±0.08$^{b}$ | 3.85±0.13$^{a}$ | 3.94±0.12$^{a}$ | 4.15±0.15$^{a}$ | 4.08±0.16$^{a}$ |
| ADG (g/day) | 0.30±0.01$^{c}$ | 0.49± 0.04$^{b}$ | 0.61±0.03$^{ab}$ | 0.70±0.06$^{a}$ | 0.59±0.05$^{ab}$ |
| FI (g feed/fish) | 48.12±0.23$^{b}$ | 51.57±0.49$^{ab}$ | 53.03±0.62$^{a}$ | 53.79±0.18$^{a}$ | 51.84±0.76$^{ab}$ |
| FCR | 2.45±0.10$^{a}$ | 1.62±0.12$^{b}$ | 1.48±0.11$^{b}$ | 1.31± 0.10$^{b}$ | 1.41±0.13$^{b}$ |
| SR (%) | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |

Results are represented as mean ± SEM (n = 4). Superscripts in each row indicate a significant difference among the treatments ($P < 0.05$). IW = Initial weight (g), FW = Final weight (g), WG = Weight gain (g), SGR = Specific growth rate (%/day), ADG = Average daily gain (g/day), FI = Feed intake (g feed/fish), FCR = Feed conversion ratio, SR = Survival rate (%).

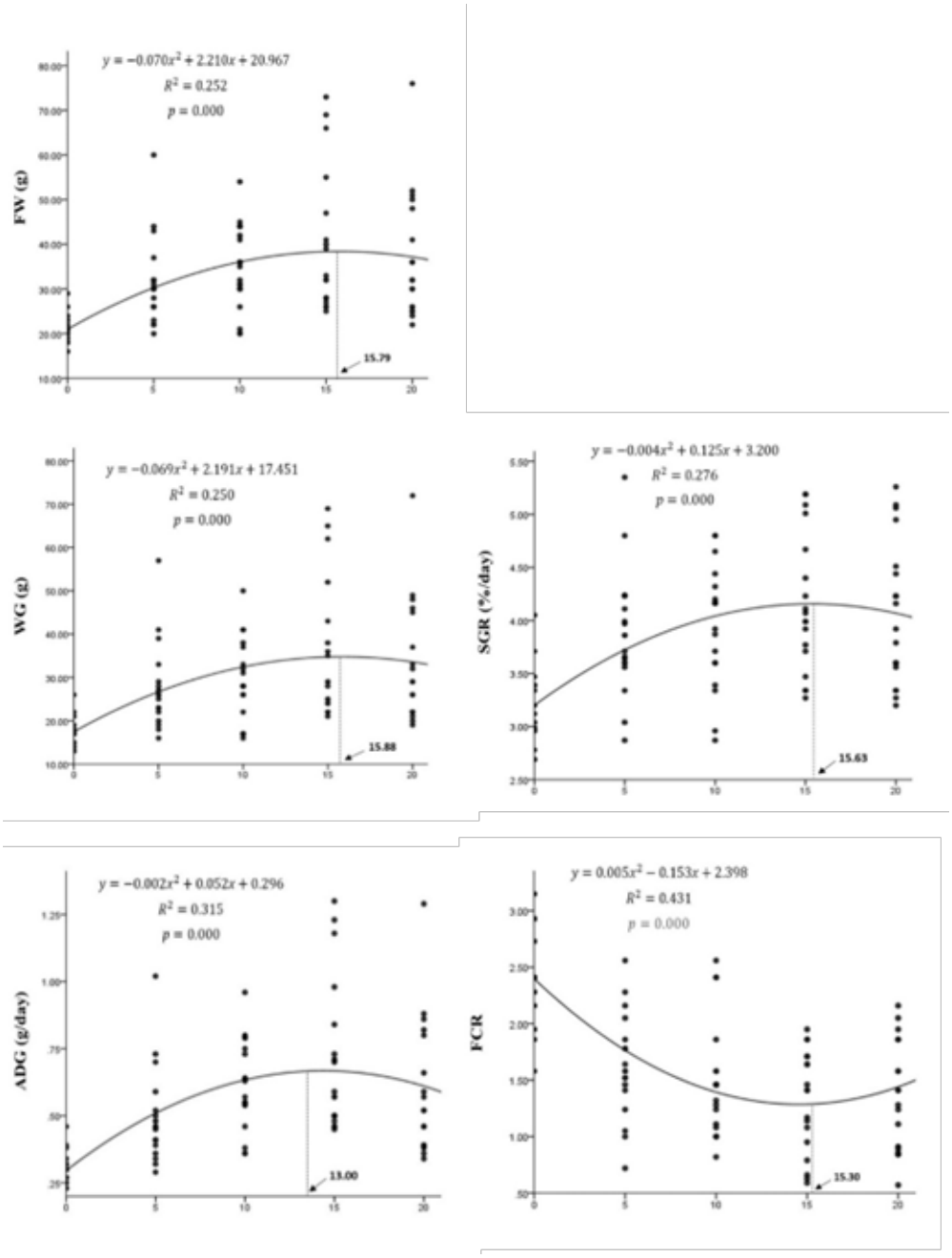


**Figure 1** The second-order polynomial regression analysis on the final weight (FW), weight gain (WG), specific growth rate (SGR), average daily gain (ADG), and feed conversion ratio (FCR) of climbing perch fed diets containing various levels of SPP for 8 weeks.

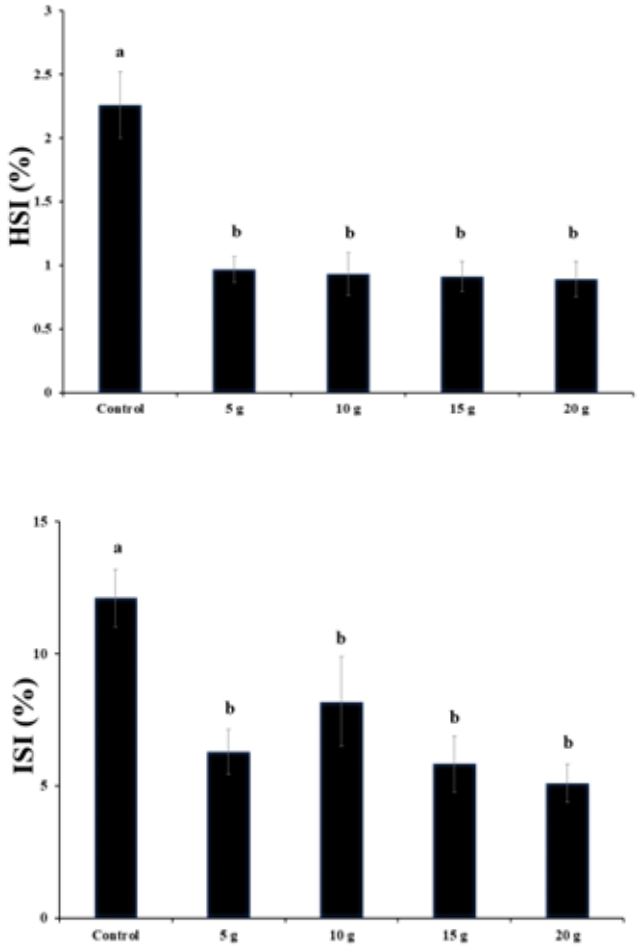


**Figure 2** Intestinosomatic and hepatosomatic indices of climbing perch fed diets containing various levels of SPP for 8 weeks. Results are represented as mean ± SEM (n = 4). Error bars with different superscripts indicate a significant difference among the treatments ($P < 0.05$). ISI = Intestinosomatic index (%), HSI = Hepatosomatic index (%).

## Digestive enzyme activities

The effects of dietary SPP on the digestive enzyme activities of climbing perch are shown in Figure 3. Amylase activity was elevated significantly in fish fed with 5 and 10 g SPP/kg diets compared with other groups ($P < 0.05$). Protease activity of fish fed with diets supplemented with 15 and 20 g SPP/kg diets was significantly increased compared with the control fish ($P < 0.05$). Fish fed the diets supplemented with 10 and 15 g SPP/kg diets showed a significant enhancement of lipase activity compared to other diets ($P < 0.05$). The trypsin activity was improved significantly in fish fed with a 10 g SPP/kg diet compared to other diets ($P < 0.05$). The pepsin activity showed a significant increase in fish fed the 5, 10, and 15 g SPP/kg diets compared to other diets ($P < 0.05$). The esterase activity was enhanced significantly in the experimental groups compared with the control group ($P < 0.05$).

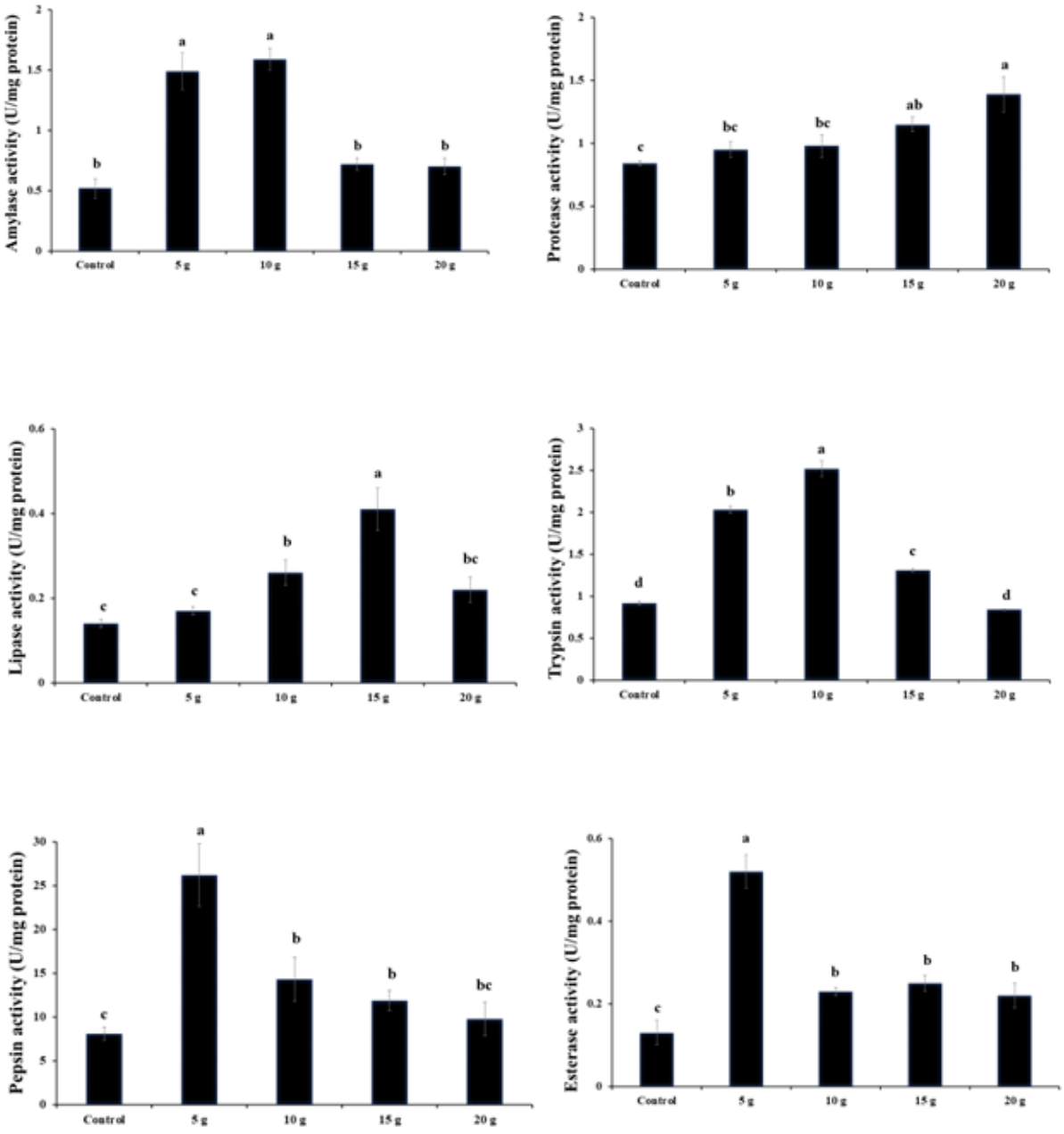


**Figure 3** Digestive enzyme activities of climbing perch fed diets containing various levels of SPP for 8 weeks. Results are represented as mean ± SEM (n = 4). Error bars with different superscripts indicate a significant difference among the treatments ($P < 0.05$).

### Mucosal immune parameters

The determined values of skin mucus immune parameters of climbing perch fed with different levels of SPP are represented in Table 4. The highest total protein level was found in fish fed with a 5 g SPP/kg diet compared to the groups fed with other diets ($P < 0.05$). IgM, lysozyme, antiprotease, and MPO levels significantly increased in fish fed with diets supplemented with SPP compared to the control fish ($P < 0.05$). Increased ALP levels were detected in fish fed with 10, 15, and 20 g SPP diets compared to other groups ($P < 0.05$). The esterase level was significantly increased in fish fed with 15 and 20 g SPP diets compared to other groups ($P < 0.05$).

**Table 4** Skin mucus immune parameters in climbing perch fed diets containing various levels of SPP for 8 weeks

| Parameters | SPP levels (g/kg diet) | | | | |
|---|---|---|---|---|---|
| | Control | 5 g | 10 g | 15 g | 20 g |
| Total protein (mg/mL) | 1.32±0.06$^{b}$ | 2.33±0.18$^{a}$ | 1.49±0.16$^{b}$ | 1.28±0.17$^{b}$ | 1.58±0.14$^{b}$ |
| Ig M (mg/mL) | 0.56±0.06$^{d}$ | 1.77±0.21$^{a}$ | 1.26±0.18$^{bc}$ | 0.91±0.15$^{c}$ | 1.46±0.73$^{ab}$ |
| Lysozyme (U/mg protein) | 3.05±0.60$^{c}$ | 9.11±1.19$^{b}$ | 11.76±1.43$^{b}$ | 27.09±1.93$^{a}$ | 15.99±1.14$^{ab}$ |
| ALP (U/mg protein) | 11.93±3.39$^{d}$ | 11.32±2.04$^{d}$ | 22.56±1.07$^{c}$ | 52.62±1.41$^{b}$ | 88.05±2.17$^{a}$ |
| Antiprotease (%) | 17.33±0.65$^{b}$ | 23.32±0.33$^{a}$ | 20.94±0.19$^{a}$ | 25.27±0.28$^{a}$ | 22.33±0.16$^{a}$ |
| Esterase (U/mg protein) | 5.13±0.20$^{b}$ | 4.71±0.48$^{b}$ | 4.84±0.47$^{b}$ | 6.76±0.33$^{a}$ | 6.44±0.61$^{a}$ |
| MPO ($OD_{450}$) | 0.59±0.04$^{e}$ | 1.39±0.03$^{d}$ | 2.00±0.2$^{b}$ | 2.72±0.01$^{a}$ | 1.57±0.00$^{c}$ |

Results are represented as mean ± SEM (n = 4). Superscripts in each row indicate a significant difference among the treatments ($P < 0.05$). Ig M = immunoglobulin M (mg/mL), ALP = alkaline phosphatase (U/mg protein), MPO = myeloperoxidase ($OD_{450}$)

### Antioxidant activities

The results of the antioxidant response in the skin mucus of fish fed with SPP containing diets are displayed in Figure 4. SOD levels were significantly increased in fish fed with SPP containing diets compared to the control group ($P < 0.05$). Additionally, the CAT level observed in fish fed with 10 and 15 g/kg diets was significantly improved compared to other groups ($P < 0.05$). Furthermore, the TAC of fish fed with 5, 10, and 15 g SPP diets was significantly enhanced compared to other groups ($P < 0.05$).

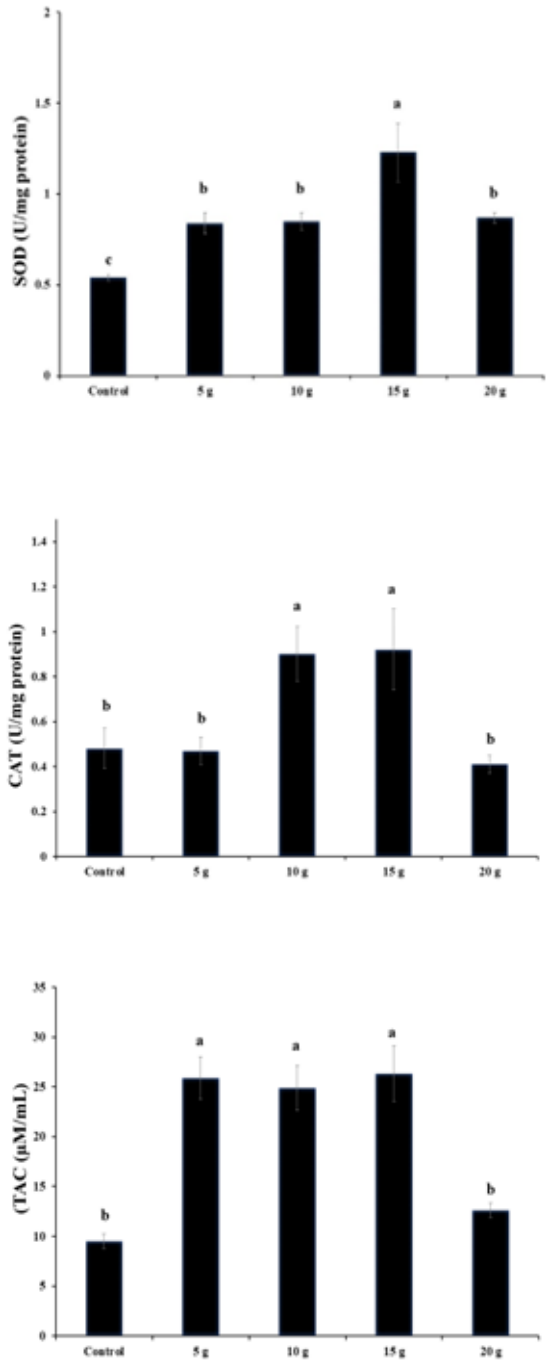


**Figure 4** Antioxidant capacity in the skin mucus of climbing perch fed diets containing various levels of SPP for 8 weeks. Results are represented as mean ± SEM (n = 4). Error bars with different superscripts indicate a significant difference among the treatments ($P < 0.05$). SOD = superoxide dismutase (U/mg protein), CAT = catalase (U/mg protein), TAC = total antioxidant capacity (µM/mL).

## DISCUSSION

The global demand for fish and their products continuously increases each year. To increase production, intensive fish culture conditions are applied (Adel et al., 2021). However, these practices can produce adverse effects on the health and physiological conditions of the cultured fish due to overcrowding, wastes, disease outbreaks, and biological interaction with flora and fauna (Chakraborty et al., 2014; Ramezani et al., 2021). The use of synthetic compounds and antibiotics to increase the health and survival of farmed fish is prohibited worldwide (Mbokane and Moyo, 2022). Investigations of the effects of different types of orally administered growth-promoting substances or immunostimulants derived from plants and natural products have been a focus of attention, and modes of action have also been explored (Akrami et al., 2015; Khunchalee and Munglue, 2020; Vijayaram et al., 2022). Therefore, this research studied the effects of dietary supplementation of SPP on the growth and physiological status of climbing perch.

The results of the phytochemical evaluation indicated that SPP contains flavonoids, terpenoids, phenolic compounds, tannins, steroids, and glycosides. These findings are inconsistent with the report of Sameh et al. (2018), who indicated that different *Spondias* species had phenolic compounds, flavonoids, steroids, triterpenoids, alkaloids, saponins, and tannins. It is well-known that factors affecting phytochemical contents in plants are geographical location, climate types, soil composition, temperature, plant diseases, and pests (Tacouri et al., 2013). Secondary metabolite content depends on plant organs, drying methods, solvents, and extraction methods (Tacouri et al., 2013). In aquaculture industry, plant extracts and their active components have been evaluated for improving growth performance and feed utilization efficiency in several aquatic animal species (Vijayaram et al., 2022). Studies have shown that natural products include antioxidant, antimicrobial, anti-inflammatory, antiviral, and immunomodulatory activities that may improve the performance, health, and overall physiological status of fish (Chakraborty et al., 2014; Reverter et al., 2014). Qualitative analysis of SPP demonstrated the presence of several bioactive compounds such as flavonoids, terpenoids, and phenolic compounds. The mean values for the total phenolic and flavonoid contents of SPP were 204.70±2.06 mg GAE/g of extract and 172.53±1.65 mg QE/g of extract, respectively. Furthermore, SPP demonstrated DPPH scavenging activity with an $IC_{50}$ of 224.64±1.81 µg/mL. Based on these findings, SPP could be considered for use as antioxidant and growth-promoting agents in aquaculture production due to its rich content of various potent phytochemicals.

The present study documented that fish fed diets supplemented with SPP significantly increased their growth and performance. In addition, the FCR of the tested fish was significantly reduced compared with the control fish. By using the second-order polynomial regression analysis, a suitable concentration level of SPP was 13.00 – 15.88 g/kg diet. An increase in the growth of fish fed with diets containing plant products may be due to the improvement in feed intake, palatability, enzyme activity, digestion and absorption processes, and nutrient utilization (Awad and Awaad, 2017). Many beneficial effects of plants, their extracts or their major active compounds on fish growth have been reported (Khunchalee and Munglue, 2020; Mbokane and Moyo, 2022). It is suggested that flavonoids, terpenoids, phenolic compounds, tannins, steroids,

and glycosides are growth-promoting substances in several aquatic animals by up-regulating growth-related gene expression (Ahmadifar et al., 2021). It is hypothesized that flavonoids, terpenoids, phenolic compounds, tannins, steroids, and glycosides found in SPP could be contributing to the enhancement of growth and feed intake observed in this research. Interestingly, the level of 20 g SPP/kg diet produced a significant decrease in growth in climbing perch. These adverse effects of SPP on fish growth may be related to anti-nutrient components, excessive doses, oxidative stress, and allergic responses. It has been noted that polyphenols and tannins in plant extracts can suppress the absorption capacity of amino acids in the intestines (Dawson et al., 1999). However, the exact mechanism of the growth-promoting effect of SPP has yet to be discovered. Further research is required to clarify how SPP enhances fish growth and to understand its impact on the expression of growth-related genes.

Supplementation of SPP significantly decreased HSI and VSI values in fish compared with the control. It is generally accepted that the measurement of organosomatic indices, such as HSI and ISI, positively indicates the nutritional response of fish fed with the tested diets (Lee et al., 2014). Decreased HSI is directly related to preventing lipid storage in hepatocytes, which would benefit fish health (Teimouri et al., 2016). Interestingly, a previous report showed that flavonoids and phenolics decreased lipid accumulation and caused lipolysis (Swathi and Lakshman, 2022). Therefore, decreased HSI would suggest the hypolipidemic action of SPP containing diet in fish, probably due to the presence of flavonoids and phenolics in SPP (Lee et al., 2014). A reduction in ISI observed would produce an economic advantage in this experiment because the fish intestines are generally discarded during the fish processing operation (Teimouri et al., 2016).

Digestive enzyme activities are indicators of digestibility and nutrient availability in fish fed with supplementary diets (Jiang et al., 2016). This study showed a significant increase in intestinal digestive enzyme activities, including lipase, amylase, protease, trypsin, pepsin, and esterase, in fish fed with SPP containing diets. This finding demonstrates a positive effect of SPP on digestibility, absorption capacity, and nutrient utilization in climbing perch, similar to that reported by Mohammadi et al. (2020). It is assumed that such phytochemicals could activate the secretion of bile acids, enhance digestive enzyme activities, and improve digestive tract functions in fish (Zhang et al., 2020; Ramezani et al., 2021). Moreover, dietary supplementation with herbs could regulate intestinal metabolisms by activating mucin secretion to form a barrier structure covering the apical surface of the absorptive cells. Jiang et al. (2016) reported that phytoconstituents can modulate intestinal bacteria to produce some essential digestive enzymes in metabolism. It is hypothesized that some bioactive principles in SPP could regulate the transcription of digestive enzyme-related genes in fish (Jiang et al., 2016; Dawood et al., 2022). Thus, SPP could improve the absorption and utilization of nutrients in cultured fish by modulating digestive enzyme activities, as observed in the present study. However, it is demonstrated that fish fed with diets containing high levels of SPP showed a marked reduction of digestive enzymes such as amylase, lipase, and trypsin. These results may be due to the inhibition of digestive enzymes by the high concentrations of polyphenolic compounds found in SPP as already reported by Martínez-Gonzalez et al. (2017). Additional investigations are

necessary to examine the influence of SPP on enzymatic activities and the expression of digestive enzyme genes in fish.

Skin mucus is one of the innate immune components found in fish. Several active components, such as antibacterial enzymes, proteins, peptides, carbohydrates, water, lipids, and metabolites, are predominant components in the mucus (Dash et al., 2018). The mucus is produced by goblet, sacciform, and club cells in the epidermal layer (Shephard, 1993; Dash et al., 2018). The primary functions of fish skin mucus are crucial for osmoregulation, action against pathogenic microorganisms, and intra- and inter-specific communication (Firmino et al., 2021). The component of skin mucus is an essential tool for evaluating the immune stimulant properties of natural feed additives in fish (Adel et al., 2021). The current study demonstrated that non-specific immune components, including total protein, IgM, lysozyme activity, ALP, antiprotease, esterase, and MPO, in the skin mucus were significantly improved in the experimental treatments compared with the control. The enhancement of the innate immune components in fish fed with the dietary application of SPP could be correlated with phytochemicals detected in SPP that showed immune-enhancing properties, such as phenolics, flavonoids, terpenoids, coumarins, and tannins (Reverter et al., 2014; Hoseinifar et al., 2020). Moreover, herbs may improve the non-specific immune system by up-regulating immune-related gene transcription in fish (Ahmadifar et al., 2021; Huang et al., 2022; Kumar et al., 2022). Thus, these results suggest that SPP could act as a natural immunomodulator in climbing perch (Reverter et al., 2014). However, further study is needed to understand the mode of action through which SPP enhances mucosal immune gene expression.

The production of reactive oxygen species (ROS) typically occurs in fish during the metabolic processes or exposure to several stressors such as pollutants, overcrowding cultivation, and xenobiotics (Habotta et al., 2022). SOD and CAT are two crucial antioxidant enzymes released from white blood cells. These enzymes play a vital role in removing ROS from cellular components in the fish body (Huang et al., 2022). Total antioxidant capacity provides the overall antioxidant defense systems in animals. In the present study, SOD, CAT, and TAC activities in fish skin mucus were significantly elevated in fish fed diets fortified with SPP compared with the control diet. This finding implied the antioxidative effects of SPP on climbing perch. It was shown that plants belonging to the genus *Spondias* possessed antioxidative properties (Sameh et al., 2018). Phytochemical compounds, especially flavonoids, phenolics, and tannins, have been reported to have free radical scavenging activities (Laksemi et al., 2019). Increased SOD, CAT, and TAC levels observed in this research are expected to be attributed to the antioxidant properties of some active ingredients found in SPP, including flavonoids, phenolics, and tannins (Habotta et al., 2022). Additionally, compounds like polyphenols could modulate antioxidant-related gene expression through specific pathways in fish (Jiang et al., 2016; Huang et al., 2022). However, the higher level of SPP (20 g/kg diet) decreased SOD, CAT, and TAC in climbing perch. The reduction in antioxidant enzyme activities in the skin mucus may be due to a loss or an impairment of the immune system produced by the high dose of SPP (Vazirzadeh et al., 2019). Hence, additional research is needed to investigate the effects of SPP on the expression of antioxidant enzyme genes in fish.

Approximately, two-thirds of the variable costs in aquaculture production are attributed to fish feeds (Parker, 2012). To enhance business profitability, aquaculturists should focus on reducing feed costs and controlling disease issues (Habotta et al., 2022). The utilization of feed additives derived from cost-effective natural products in fish production, aimed at improving aquaculture nutrition and health, represents one approach to achieving these objectives and increasing profits. In light of the findings from this research, it is recommended that the application of SPP in aquafeed be implemented proactively, prior to the onset of pathogenic challenges and disease outbreaks, at the optimal doses as recommended. This approach can help enhance the overall health and physiological conditions of cultured fish and subsequently reduce mortality rates (Awad and Awaad, 2017; Habotta et al., 2022).

## CONCLUSIONS

In conclusion, the current study revealed that SPP-containing diets had a growth-promoting effect on climbing perch. However, ISI and HSI values reduced significantly in fish fed dietary supplementation of SPP. Digestive enzyme activities, skin mucus immunity, and antioxidant activities of fish fed with SPP were significantly enhanced. The mode of action of SPP on growth and physiological aspects of fish observed in this experiment might be attributed to antioxidant property and some phytochemicals detected in SPP such as flavonoids and phenolic compounds. Thus, SPP could be useful for developing a feed additive with the potential to improve the growth performance and health status of the climbing perch. The suitable level of SPP estimated using the second-order polynomial regression analysis was 13.00 – 15.88 g SPP/kg diet.

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## AUTHOR CONTRIBUTIONS

Conceptualization, investigation, and validation were performed by Wilawan Promprom. Data collection was conducted by Wannachai Chatan and Somsak Khambaione. Statistical analysis was done by Kajita Somnate. Conceptualization, study design, data collection and analysis, and writing-original draft were performed by Phukphon Munglue. All authors have read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare that they have no known competing interests that could have appeared to influence the work reported in this paper.

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