Effects of Spatholobus suberectus Ethanol Extract on Antioxidant Capacity, Non-specific Immunity and Disease Resistance of Yellow Catfish (Pelteobagrus fulvidraco) Postprint

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Abstract

To investigate the effects of Spatholobus suberectus ethanol extract on the antioxidant capacity, non-specific immunity, and disease resistance of yellow catfish (Pelteobagrus fulvidraco), 720 healthy one-year-old all-male yellow catfish with initial body weight of (35.25±1.63) g and initial body length of (14.07±0.24) cm were selected and randomly divided into 6 groups (3 replicates per group, 40 fish per replicate), fed isonitrogenous and isoenergetic diets supplemented with 0, 0.04%, 0.08%, 0.16%, 0.32%, and 0.64% Spatholobus suberectus ethanol extract, respectively labeled as groups T1–T6, with group T1 serving as the control group. After 60 days of feeding, the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) and the contents of malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO) in various tissues (gill, brain, head kidney, trunk kidney, hepatopancreas, spleen) and serum of yellow catfish were determined, as well as the activities of alanine aminotransferase (GPT) and aspartate aminotransferase (GOT) in hepatopancreas and serum, and the contents of complement 3 (C3), immunoglobulin (IgM), and albumin (ALB) in serum. After the feeding trial, a challenge test was conducted by injecting Aeromonas hydrophila, and the cumulative mortality and immune protection rate were calculated 7 days post-challenge. The results showed that: dietary supplementation with 0.16%–0.32% Spatholobus suberectus ethanol extract significantly increased the activities of SOD, CAT, and GSH-Px and the content of GSH in various tissues and serum, as well as the contents of C3 and ALB in serum ($P<0.05$), and significantly decreased the MDA content in various tissues and serum of yellow catfish ($P<0.05$); the activities of GOT and GPT in hepatopancreas showed a trend of first increasing and then decreasing with increasing supplementation levels of Spatholobus suberectus ethanol extract,

while the opposite trend was observed in serum; no significant differences were observed in serum IgM content among all groups (P>0.05); the NO content in hepatopancreas, serum, and head kidney showed a trend of first increasing and then decreasing with increasing supplementation levels of Spatholobus suberectus ethanol extract, while the NO content in gill and brain showed a trend of first decreasing and then increasing; the NO content in spleen and trunk kidney did not change significantly with varying supplementation levels of Spatholobus suberectus ethanol extract (P>0.05); in the challenge test, dietary supplementation with 0.16%-0.32% Spatholobus suberectus ethanol extract significantly reduced the cumulative mortality 7 days post-challenge (P<0.05) and effectively increased the immune protection rate. Therefore, based on comprehensive consideration of antioxidant capacity, non-specific immunity, and disease resistance, the optimal dietary supplementation level of Spatholobus suberectus ethanol extract for yellow catfish is 0.16%-0.32%.

Full Text

Effects of Caulis spatholobi Ethanol Extract on Antioxidant Capacity, Non-Specific Immunity, and Disease Resistance of Yellow Catfish (Pelteobagrus fulvidraco)

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Abstract

This study investigated the effects of Caulis spatholobi ethanol extract on the antioxidant capacity, non-specific immunity, and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). Seven hundred twenty healthy one-year-old all-male yellow catfish with an initial body weight of (35.25±1.63) g and initial body length of (14.07±0.24) cm were randomly divided into six groups (three replicates per group, 40 fish per replicate). The fish were fed isonitrogenous and isoenergetic diets supplemented with 0, 0.04%, 0.08%, 0.16%, 0.32%, and 0.64% Caulis spatholobi ethanol extract, designated as groups T1–T6, with T1 serving as the control. After 60 days of feeding, we measured superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities, as well as malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO) contents in various tissues (gill, brain, head kidney, middle kidney, hepatopancreas, spleen) and serum. Additionally, we determined glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities in hepatopancreas and serum, and complement 3 (C3), immunoglobulin M (IgM), and albumin (ALB) contents in serum.

Following the feeding trial, a challenge test was conducted by injecting *Aeromonas hydrophila*, and cumulative mortality and immune protection rate were calculated seven days post-challenge. The results demonstrated that dietary supplementation with 0.16%–0.32% Caulis spatholobi ethanol extract significantly enhanced SOD, CAT, and GSH-Px activities and GSH content in tissues and serum ($P<0.05$), significantly increased serum C3 and ALB contents ($P<0.05$), and significantly reduced MDA content in tissues and serum ($P<0.05$). GOT and GPT activities in hepatopancreas increased initially then decreased with increasing extract levels, while showing the opposite trend in serum. No significant differences were observed in serum IgM content among groups ($P>0.05$). NO content in hepatopancreas, serum, and head kidney increased initially then decreased with supplementation, whereas NO in gill and brain decreased initially then increased; NO content in spleen and middle kidney remained unchanged ($P>0.05$). In the challenge test, 0.16%–0.32% Caulis spatholobi ethanol extract significantly reduced cumulative mortality ($P<0.05$) and effectively improved immune protection rate. Based on comprehensive evaluation of antioxidant capacity, non-specific immunity, and disease resistance, the optimal dietary inclusion level of Caulis spatholobi ethanol extract for yellow catfish is 0.16%–0.32%.

Keywords: *Pelteobagrus fulvidraco*; Caulis spatholobi ethanol extract; antioxidant capacity; non-specific immunity; disease resistance

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Introduction

Caulis spatholobi is a traditional Chinese medicine rich in flavonoids, primarily distributed in Guangdong, Guangxi, and Yunnan provinces. Modern pharmacological research has demonstrated its antioxidant, antiviral, antitumor, and lipid metabolism-regulating properties. The ethanol extract of Caulis spatholobi contains complex components, mainly including flavonoids, lignans, and anthraquinones, with flavonoids serving as the marker compounds. Flavonoids are classified into flavones, isoflavones, and other categories, comprising compounds such as formononetin, ononin, and prunetin. These compounds exhibit significant efficacy in eliminating free radicals and inhibiting bacteria and cancer, with no side effects, offering broad research prospects. Flavonoids exert antioxidant effects on lipids through two pathways: first, by eliminating the catalytic effects of metal ions such as iron and copper; second, by donating hydrogen to lipid free radicals, transforming into phenoxy radicals that stabilize and reduce the propagation rate of auto-oxidation chain reactions, thereby inhibiting further lipid oxidation.

Yellow catfish (*Pelteobagrus fulvidraco*), belonging to Siluriformes and the genus *Pelteobagrus*, is widely distributed across various water systems and represents an economically important fish species. Its meat is tender, delicious, and free of intermuscular bones, with high flesh content, moderate fat content, abundant

minerals, good water-holding capacity, and desirable textural properties of firmness and elasticity. Consequently, it has become a popular aquaculture species and premium market product in China. In Tianjin, yellow catfish sales are substantial, with abundant aquaculture resources and high economic efficiency in large-pond polyculture systems. However, high-density farming in recent years has led to frequent disease outbreaks, high mortality rates, and severe economic losses, constraining the healthy and sustainable development of yellow catfish aquaculture. Therefore, this study investigated the effects of Caulis spatholobi ethanol extract on antioxidant capacity, non-specific immunity, and disease resistance in yellow catfish, aiming to identify novel additives that could enhance antioxidant capacity and non-specific immunity while reducing disease incidence, thereby providing reference for developing immunostimulants for yellow catfish aquaculture.

Materials and Methods

Experimental Fish Healthy one-year-old all-male yellow catfish were obtained from Tianjin Lanke Aquatic Co., Ltd., with initial body weight of (35.25±1.63) g and initial body length of (14.07±0.24) cm. Prior to the experiment, fish were acclimated for 30 days in indoor culture facilities, fed a basal diet to adapt to the feed and environment, with daily water exchange of one-third volume. A total of 720 healthy yellow catfish were selected for the formal experiment.

Experimental Diets Feed ingredients were purchased from Tianjin Tianxiang Aquatic Co., Ltd. All ingredients were ground and passed through an 80-mesh sieve before mixing. Distilled water was added to achieve a consistency that could form pellets without crumbling. The mixture was processed using a flat-die pelletizer to produce 2.5 mm diameter pellets, which were oven-dried for 30 minutes, then air-dried in a shaded area and stored in a dry, dark location to prevent spoilage. Caulis spatholobi ethanol extract (purchased from Xi' an Ruilin Biotechnology Co., Ltd.) was added at levels of 0 (control), 0.04%, 0.08%, 0.16%, 0.32%, and 0.64%, designated as groups T1–T6. The composition and nutritional levels of experimental diets are presented in .

TABLE 1 Composition and nutrient levels of experimental diets (air-dry basis)

Items	Groups
Ingredients	
Fish meal	
Soybean meal	
Peanut meal	
Cottonseed meal	
Canola meal	
Soybean oil	

Items	Groups
Premix[1])	
Flour	
Bran	
Caulis spatholobi ethanol extract	
Carboxymethyl cellulose	
Total	
Nutrient levels[2])	
Crude protein (CP)	
Crude lipid (EE)	
Crude ash (Ash)	
Moisture	
Total phosphorus (TP)	
Gross energy (GE) (MJ/kg)	

[1]) Premix provided the following per kg of diet: Fe 150 mg, Zn 30 mg, Mn 13 mg, Cu 3 mg, Co 0.1 mg, I 0.6 mg, Se 0.15 mg, VC 100 mg, VB 3 mg, VB 10 mg, VB 12 mg, calcium pantothenate 30 mg, nicotinic acid 30 mg, biotin 0.1 mg, folic acid 2 mg, VB 0.01 mg, inositol 400 mg, choline 1,000 mg, VA 2,000 IU, VD 1,000 IU, VE 60 mg, VK 6 mg.

[2]) GE was a calculated value, while the others were measured values.

Feeding Trial The 720 yellow catfish were distributed into 18 circular incubation tanks (1.5 m diameter, 0.75 m depth) at Tianjin Lanke Aquatic Co., Ltd. hatchery facility, with six treatment groups and three replicates per group (40 fish per replicate). Tanks were labeled T1-1 through T6-3 and fed corresponding diets. Aerators operated 24 hours daily. Fish were fed twice daily at 09:00 and 17:00. Water exchange was performed daily in the afternoon, alternating between one-third and two-thirds volume replacement to maintain stable water quality and prevent disease. The feeding trial lasted 60 days, with body length, height, and weight recorded before and after the trial for growth parameter determination.

Sample Collection At the end of the feeding trial, 15 fish per replicate were randomly selected for sampling. Blood was collected from the caudal vein using 2 mL sterile syringes, centrifuged at 4,500 r/min for 15 minutes to obtain serum samples, which were stored at -80°C for analysis. After blood collection, fish were dissected on ice. Gill, brain, head kidney, middle kidney, hepatopancreas, and spleen were removed and rinsed with physiological saline to remove blood clots and adipose tissue. Tissues were homogenized in physiological saline at a 1:9 mass-to-volume ratio to prepare 10% homogenates (intestine at 1:4 ratio for 20% homogenate). Homogenates were centrifuged at 4,000 r/min for 20 minutes, and supernatants were collected and stored at -80°C.

Analytical Methods Feed Nutrient Analysis
Moisture content was determined by oven-drying at 105°C (GB/T 5009.3-2010). Crude protein (CP) was measured using the Dumas combustion method (GB/T 24318-2009). Crude lipid (EE) was extracted using the Soxhlet method (GB/T 5009.6-2010). Crude ash was determined by incineration at 550°C (GB/T 5009.4-2010). Calcium (Ca) content was measured by potassium permanganate titration (GB/T 6436-2002). Total phosphorus (TP) was determined by molybdovanadate colorimetry (GB/T 6437-2002). Total sugar (TS) was measured by potassium ferricyanide method (GB/T 9695.31-1991). Gross energy (GE) was calculated using the formula: GE = (CP×5.64 + EE×9.44 + TS×4.11)/100 × 4.184.

Antioxidant and Non-Specific Immune Indices
Assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-Px), nitric oxide (NO), glutamic-pyruvic transaminase (GPT), glutamic-oxaloacetic transaminase (GOT), complement 3 (C3), immunoglobulin M (IgM), and albumin (ALB) were purchased from Nanjing Jiancheng Bioengineering Institute. All measurements were performed according to manufacturer instructions.

Disease Resistance Assessment
Following the feeding trial, a challenge test was conducted using *Aeromonas hydrophila* preserved at Tianjin Fisheries Research Institute. Bacteria were activated and diluted with sterile phosphate-buffered saline (PBS) to a concentration of 2×10 CFU/mL. Ten fish per tank with uniform body length and weight were intraperitoneally injected with 2 mL bacterial suspension per 100 g body weight. Culture conditions remained unchanged, and mortality was recorded daily for one week. Cumulative mortality and immune protection rate were calculated as follows:

Cumulative mortality (%) = 100 × N /N
Immune protection rate (%) = (1 - F /F) × 100

Where N = initial number of infected fish, N = final number of dead fish, F = mortality rate of control group, and F = mortality rate of treatment group.

Data Processing and Analysis Experimental data are expressed as mean ± standard error. One-way ANOVA and LSD multiple comparison tests were performed using SPSS 16.0 software, with $P<0.05$ considered statistically significant.

Results

Effects of Caulis spatholobi Ethanol Extract on SOD Activity As shown in , SOD activity in control group fish followed the order: hepatopancreas > middle kidney > spleen > gill > head kidney > serum > brain. This pattern remained largely unchanged after extract supplementation, except for position exchanges between gill and head kidney in T3, T5, and T6 groups. Compared

with the control, T2 group showed elevated SOD activity in all tissues and serum, but differences were not significant (P>0.05). T3 group exhibited significantly increased SOD activity in middle kidney, spleen, and serum (P<0.05), reaching 1.68, 1.58, and 1.33 times control values, respectively. T4 group showed significant enhancement of SOD activity in all examined tissues and serum (P<0.05), with values 1.51, 2.37, 1.92, 1.81, 1.73, 1.48, and 1.70 times higher than control in hepatopancreas, middle kidney, spleen, gill, head kidney, serum, and brain, respectively. T5 group also demonstrated significantly increased SOD activity in hepatopancreas, middle kidney, spleen, gill, and head kidney (P<0.05), at 1.30, 2.03, 1.83, 1.34, and 1.43 times control values.

TABLE 2 Effects of Caulis spatholobi ethanol extract on SOD activity of *Pelteobagrus fulvidraco* (U/mg prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	187.56±5.67a	220.82±2.23bc	230.04±6.82bc	283.14±3.36d	243.87±9.42ab	231.59±17.24bc
Head kidney	66.23±6.41a	71.93±12.96bc	86.72±1.71bc	104.59±0.08a	85.01±16.88ab	91.88±0.36bc
Spleen	54.46±0.39a	65.67±2.99a	72.20±4.01ab	80.94±2.45a	70.32±3.06a	62.28±9.42bc
Gill	66.30±7.30a	73.57±10.28a	70.86±0.20a	120.10±0.78b	88.53±2.96ab	71.31±7.72c
Mid kidney	29.36±1.36a	36.47±7.64ab	39.29±7.62ab	50.11±2.54b	44.63±2.70ab	36.38±6.97ab
Serum	83.70±5.15a	97.72±5.59bc	111.87±2.28ab	147.47±20.25d	122.95±20.92b	118.84±7.92ab
Brain	104.54±0.48a	153.19±16.37bc	176.13±0.32b	247.64±45.23d	232.51±39.20cd	209.66±2.90ab

In the same row, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), different lowercase letters indicate significant difference (P<0.05), and different capital letters indicate extremely significant difference (P<0.01). The same applies below.

Effects of Caulis spatholobi Ethanol Extract on MDA Content As presented in , MDA content in control group fish followed the order: spleen < middle kidney < gill < head kidney < hepatopancreas < serum < brain. T2, T5, and T6 groups maintained this pattern, while T3 and T4 groups showed slight variations: middle kidney < spleen < gill < head kidney < hepatopancreas < serum < brain. Compared with control, T2 group showed significantly reduced MDA content in head kidney and spleen (P<0.05), decreasing by 23.9% and 17.8%, respectively. T3 group exhibited significant reductions in head kidney, serum, spleen, and middle kidney (P<0.05), with decreases of 29.9%, 47.9%, 27.8%, and 38.9%, respectively. T4 group demonstrated significant decreases in head kidney, serum, brain, spleen, and middle kidney (P<0.05), reducing by 60.5%, 56.0%, 48.8%, 45.0%, and 59.1%, respectively. T5 group showed significant reductions in all examined tissues (P<0.05), with hepatopancreas, head kidney, serum, gill, brain, spleen, and middle kidney decreasing by 60.6%, 38.9%, 42.1%, 59.7%, 54.7%, 54.4%, and 45.3%, respectively. T6 group exhibited significant decreases in head kidney, gill, brain, spleen, and middle kidney, with reductions of 37.4%, 54.6%, 46.1%, 33.9%, and 38.9%, respectively.

TABLE 3 Effects of Caulis spatholobi ethanol extract on MDA content of *Pelteobagrus fulvidraco* (nmol/mg prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	7.51±1.21a	6.47±0.03ab	5.83±0.66ab	5.00±0.23ab	2.96±0.41c	5.22±0.15ab
Head kidney	6.28±0.26a	4.78±1.12bc	4.40±0.22bc	2.48±0.18c	3.84±1.30bc	3.93±0.10bc
Serum	15.60±1.27a	12.01±4.43ab	13.13±1.27ab	6.87±1.37c	9.03±1.69bc	11.19±2.85ab
Brain	2.95±0.02a	1.91±0.27ab	1.87±1.03ab	1.52±0.06ab	1.19±0.17c	1.34±0.14bc
Spleen	22.08±3.56a	18.53±3.14ab	15.47±5.48ab	11.31±0.80bc	10.00±0.41c	11.90±1.94bc
Mid kidney	1.80±0.15a	1.48±0.02b	1.30±0.13b	0.99±0.12bc	0.82±0.08c	1.19±0.43b
Gill	2.03±0.36a	1.56±0.17ab	1.24±0.21bc	0.83±0.05c	1.11±2.06bc	1.24±0.09bc

Effects of Caulis spatholobi Ethanol Extract on CAT Activity According to , CAT activity in control, T2, and T3 groups followed the order: hepatopancreas > brain > gill > serum > middle kidney > spleen > head kidney. T4 and T5 groups showed the pattern: brain > hepatopancreas > gill > serum > middle kidney > spleen > head kidney. T6 group exhibited the order: brain > hepatopancreas > serum > gill > middle kidney > spleen > head kidney. T4 group showed significantly increased CAT activity in hepatopancreas, head kidney, gill, brain, and spleen ($P<0.05$), reaching 1.45, 1.77, 1.81, 2.17, and 1.43 times control values, respectively. T5 group demonstrated significant increases in head kidney and brain ($P<0.05$), at 1.67 and 1.94 times control values, respectively.

TABLE 4 Effects of Caulis spatholobi ethanol extract on CAT activity of *Pelteobagrus fulvidraco* (U/mg prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	188.05±6.42c	213.39±10.22bc	228.16±31.27bc	271.79±14.26a	262.17±15.69ab	249.69±7.47c
Head kidney	4.21±0.40c	5.73±1.12bc	6.35±0.07ab	7.47±0.07a	7.01±1.49ab	6.42±0.11ab
Serum	60.03±0.26c	72.13±6.90b	75.47±3.32ab	78.14±0.45a	76.69±1.85a	72.27±2.11a
Brain	66.30±7.30c	73.57±10.28bc	80.86±0.20ab	120.10±0.78a	88.53±2.96ab	81.31±7.72bc
Spleen	152.83±2.75c	179.67±14.29bc	208.32±3.13ab	232.28±1.22a	206.79±7.72ab	231.19±26.99b
Mid kidney	6.96±0.64c	7.32±0.07bc	8.28±0.49bc	10.02±0.80a	8.36±1.08bc	8.38±0.30abc
Gill	18.71±2.37a	23.75±3.93a	17.86±0.30a	19.71±1.69a	20.77±1.06a	23.15±1.72a

Effects of Caulis spatholobi Ethanol Extract on GSH Content As shown in , GSH content in control and T2 groups followed the order: serum > hepatopancreas > middle kidney > brain > spleen > head kidney > gill. T3, T4, T5, and T6 groups exhibited the pattern: serum > hepatopancreas > brain > middle kidney > spleen > head kidney > gill. Compared with control, all treatment groups showed significantly increased GSH content in hepatopancreas and serum, which increased initially then decreased with extract dosage, peaking

at T4 in hepatopancreas and spleen (2.09 and 2.25 times control, respectively) and at T5 in serum and brain (2.51 and 3.18 times control, respectively). Caulis spatholobi ethanol extract had no significant effect on GSH content in head kidney and gill (P>0.05).

TABLE 5 Effects of Caulis spatholobi ethanol extract on GSH content of *Pelteobagrus fulvidraco* (mol/g prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	50.08±2.50	65.00±1.48	77.12±4.71	104.63±9.53	97.75±6.14	66.50±1.06b
Head kidney	11.40±2.65	12.47±0.97	12.94±0.32	13.90±0.66	11.19±0.72	11.09±0.20
Serum	126.55±7.19	182.17±23.22	251.00±15.27	273.39±1.13	317.51±21.26	202.70±13.82b
Brain	9.02±0.43	9.05±2.05	9.45±0.40	9.37±0.87	9.66±0.78	9.37±0.08
Spleen	16.29±0.83	20.10±2.44	36.72±9.36	41.17±0.42	52.08±0.45	44.48±3.40ab
Mid kidney	11.91±0.72	13.35±0.92	17.48±0.14	26.82±3.59	21.06±1.93	16.71±0.32bc
Gill	22.99±2.61	27.46±2.50	35.06±1.87	45.77±1.12	38.13±6.96	33.32±1.56bc

Effects of Caulis spatholobi Ethanol Extract on GSH-Px Activity According to , GSH-Px activity in control group fish followed the order: head kidney > hepatopancreas > spleen > middle kidney > brain > serum > gill. T2 group showed the pattern: head kidney > hepatopancreas > brain > spleen > serum > middle kidney > gill. T3, T5, and T6 groups exhibited: head kidney > hepatopancreas > brain > serum > middle kidney > spleen > gill. T4 group displayed the order: head kidney > hepatopancreas > serum > brain > middle kidney > spleen > gill. T4 group showed significantly increased GSH-Px activity in serum and middle kidney (P<0.05), reaching 2.53 and 1.72 times control values, respectively. T5 group demonstrated significant enhancement in hepatopancreas, serum, and brain (P<0.05), at 1.79, 2.47, and 2.83 times control values, respectively. Caulis spatholobi ethanol extract had no significant effect on GSH-Px activity in head kidney, gill, or spleen (P>0.05).

TABLE 6 Effects of Caulis spatholobi ethanol extract on GSH-Px activity of *Pelteobagrus fulvidraco* (mol/g prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	74.58±15.98	84.84±8.22	102.16±8.08	127.64±17.63	185±25.03	89.43±22.99abc
Head kidney	185.32±15.10	190.86±1.09	187.28±7.38	191.76±9.11	196.71±3.53	197.60±6.39
Serum	19.78±1.86	26.37±0.01	34.29±3.73	50.11±0.02	48.79±4.24	42.20±11.19ab
Brain	11.57±0.76	12.12±0.69	13.44±0.37	12.24±1.74	11.56±0.84	12.65±1.24
Spleen	20.98±5.80	33.70±7.17	37.18±3.04	48.49±2.17	59.30±2.89	44.48±6.93ab
Mid kidney	29.02±3.53	30.44±2.56	20.78±4.02	22.92±0.26	28.78±4.19	23.01±2.29
Gill	21.19±0.22	25.59±5.39	26.11±5.11	36.36±0.20	33.31±1.35	23.39±1.73c

Effects of Caulis spatholobi Ethanol Extract on NO Content As shown in , NO content in control group fish followed the order: gill > serum > spleen > brain > hepatopancreas > middle kidney > head kidney. T2 and T3 groups exhibited: serum > gill > spleen > hepatopancreas > brain > head kidney > middle kidney. T4 group showed: serum > gill > spleen > hepatopancreas > head kidney > brain > middle kidney. T5 group displayed: serum > spleen > hepatopancreas > gill > brain > head kidney > middle kidney. T6 group demonstrated: serum > spleen > gill > brain > hepatopancreas > head kidney > middle kidney. NO content in hepatopancreas, head kidney, and serum increased initially then decreased with extract dosage, but all treatment groups showed elevated levels compared with control, with T4 group reaching 2.55 and 3.29 times control values in hepatopancreas and serum ($P<0.05$), and T5 group achieving 2.68 and 4.00 times control values in hepatopancreas and head kidney ($P<0.05$). In contrast, NO content in gill and brain decreased initially then increased, with all treatment groups showing lower levels than control, particularly in T5 gill and T4 brain, which decreased by 80.1% and 71.1%, respectively ($P<0.05$). Caulis spatholobi ethanol extract had no significant effect on NO content in spleen or middle kidney ($P>0.05$).

TABLE 7 Effects of Caulis spatholobi ethanol extract on NO content of *Pelteobagrus fulvidraco* (mol/g prot)

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas	0.22±0.04c	0.42±0.08ab	0.39±0.05b	0.56±0.09a	0.59±0.06a	0.33±0.05bc
Head kidney	0.08±0.01c	0.15±0.03bc	0.10±0.02bc	0.25±0.01b	0.32±0.01a	0.23±0.01b
Serum	1.34±0.29c	2.40±0.08b	1.91±0.32bc	4.41±0.65a	2.33±0.02b	2.69±0.08b
Brain	2.84±0.48a	1.16±0.59b	1.03±0.37bc	0.98±0.15bc	0.54±0.06c	0.73±0.30bc
Spleen	0.52±0.15a	0.31±0.03bc	0.21±0.06bc	0.15±0.01c	0.36±0.04ab	0.35±0.08abc
Mid kidney	1.28±0.24	1.11±0.10	0.93±0.01	0.95±0.12	1.14±0.48	1.20±0.24
Gill	0.13±0.02	0.12±0.05	0.07±0.03	0.10±0.03	0.03±0.01	0.09±0.03

Effects on Aminotransferase Activities As shown in , both aminotransferases exhibited higher activity in hepatopancreas than in serum. In hepatopancreas, GOT and GPT activities increased initially then decreased with extract dosage, peaking in T5 group at 1.31 and 1.21 times control values ($P<0.05$). In serum, both enzymes showed the opposite trend, decreasing initially then increasing, with minimum values in T4 (GOT) and T5 (GPT) groups, representing 41.5% and 56.3% reductions from control ($P<0.05$).

TABLE 8 Effects of Caulis spatholobi ethanol extract on aminotransferase activities in hepatopancreas and serum of *Pelteobagrus fulvidraco*

Items	T1	T2	T3	T4	T5	T6
Hepatopancreas (U/g prot)						
GOT	104.71±4.51a	113.31±34.12bc	125.11±11.73c	130.25±8.25c	97.48±13.02a	106.66±7.58b
GPT	121.22±7.89a	93.88±9.01b	134.63±8.52c	143.42±7.81c	146.43±15.73c	137.53±17.24b
Serum (U/L)						
GOT	22.26±3.52a	19.00±1.64ab	19.75±10.97ab	13.03±1.51b	17.64±9.51b	17.35±0.20bc
GPT	8.45±3.95a	6.09±2.24ab	6.42±2.92ab	3.92±0.01b	3.69±1.60b	6.07±2.11ab

Effects on Serum C3, ALB, and IgM Contents As presented in , serum C3 and ALB contents increased initially then decreased with extract dosage, reaching maximum values in T5 group at 3.51 and 1.42 times control levels ($P<0.05$). No significant differences were observed in serum IgM content among groups ($P>0.05$).

TABLE 9 Effects of Caulis spatholobi ethanol extract on C3, ALB, and IgM contents in serum of *Pelteobagrus fulvidraco*

Items	T1	T2	T3	T4	T5	T6
C3 (mg/L)	61.79±1.75a	85.19±5.47c	130.54±2.97e	173.12±8.36f	257.78±2.06g	159.09±7.00b
ALB (g/L)	26.57±4.51a	26.16±1.42a	29.55±0.46ab	33.81±2.75b	37.72±2.71b	31.96±3.59ab
IgM (mg/L)	6.97±0.61	6.66±0.58	7.02±0.70	6.48±0.35	6.93±1.05	7.24±1.17

Effects on Disease Resistance As shown in , cumulative mortality seven days post-challenge decreased initially then increased with extract dosage, reaching minimum values in T4 and T5 groups, which were 37.9% and 34.5% lower than control ($P<0.05$). Immune protection rate showed the opposite trend, increasing initially then decreasing, with maximum value in T4 group, significantly higher than T2 and T3 ($P<0.05$) but not significantly different from T5 and T6 ($P>0.05$).

TABLE 10 Effects of Caulis spatholobi ethanol extract on disease resistance of *Pelteobagrus fulvidraco*

Items	T1	T2	T3	T4	T5	T6
Cumulative mortality (%)	72.5±3.5a	62.5±3.5ab	57.5±3.5ab	45.0±7.07b	47.5±3.5b	52.5±3.5ab
Immune protective rate (%)	7.41±5.24c	14.81±5.24bc	33.33±10.48ab	49.63±5.24a	42.22±5.24ab	

Discussion

Effects on Antioxidant Capacity Caulis spatholobi is primarily applied for antioxidant, anti-inflammatory, antitumor, and sedative-hypnotic effects. Flavonoids, as one of the main active components in Caulis spatholobi ethanol extract, reportedly exert antitumor effects by regulating cell cycle progression and antioxidant effects by scavenging hydroxyl radicals (· OH), superoxide anion radicals (O ·), and hydrogen peroxide (H O). Natural flavonoids possess strong antioxidant and anti-aging properties, with hydroxyl substituents on their basic skeleton serving as active groups for free radical scavenging. The position and substitution pattern of hydroxyl groups significantly influence their biological activity. The mechanism involves ionizing hydrogen atoms to neutralize oxygen radicals and forming dimers with ionized flavonoids to prevent reverse combination, thereby eliminating free radicals. For instance, Yu et al. reported that four medicinal plant flower extracts exhibited reducing capacity positively correlated with flavonoid content within a certain concentration range.

In this study, Caulis spatholobi ethanol extract enhanced SOD, CAT, and GSH-Px activities and GSH content while significantly reducing MDA content in yellow catfish serum and tissues, indicating improved antioxidant capacity and reduced oxidative damage. SOD is a crucial antioxidant enzyme and oxygen radical scavenger; higher activity represents stronger free radical elimination capacity. CAT decomposes H O into water and oxygen through electron transfer, serving as a key enzyme in the biological defense system. GSH-Px catalyzes GSH conversion to oxidized glutathione (GSSG), reducing toxic peroxides to harmless hydroxyl compounds to protect cell membrane structure and function. Higher GSH content and GSH-Px activity indicate stronger antioxidant defense. MDA content reflects the severity of free radical attack on cells; lower values indicate reduced oxidative damage. Liu et al. found that 13.6–217.0 g/L total flavonoids from Caulis spatholobi ethanol extract concentration-dependently inhibited MDA generation in rat heart, liver, and kidney induced by Fe^{2} plus ascorbic acid, consistent with our findings. Similarly, Wang et al. reported that total flavonoids from litchi seed reduced NO and MDA contents and NOS activity while increasing SOD activity and GSH-Px content in rat intestinal tissue, paralleling our results.

Inducible nitric oxide synthase (iNOS) is a key enzyme mediating inflammatory responses, catalyzing L-arginine oxidation to NO. NO functions in intracellular signal transduction, platelet aggregation, immune defense, and vasodilation. Reaction between NO and O generates strong oxidants and peroxynitrite. Fu et al. found that the ethyl acetate fraction of Caulis spatholobi flavonoids significantly reduced NO secretion and intracellular reactive oxygen species (ROS) in H O -induced oxidative stress cells, demonstrating protective effects against oxidative damage. In this study, Caulis spatholobi ethanol extract increased NO content in hepatopancreas, head kidney, and serum while decreasing NO in gill and brain, which are intimately involved in oxygen exchange. These results align with previous findings, suggesting that increased NO in hepatopancreas,

head kidney, and serum enhances antioxidant capacity, while decreased NO in gill and brain reduces damage from strong oxidants and peroxynitrite.

Although flavonoid research is relatively extensive, studies on flavonoids extracted from Caulis spatholobi regarding antioxidant capacity remain limited, particularly in yellow catfish. Our results demonstrate that Caulis spatholobi ethanol extract improves antioxidant capacity in yellow catfish, warranting further molecular-level investigation of its mechanisms.

Effects on Non-Specific Immunity and Disease Resistance Fish possess both specific and non-specific immunity, but compared with mammals, their specific immune mechanisms are less developed, relying primarily on non-specific immunity. Transaminases, including GOT and GPT, are important enzymes in amino acid metabolism. In healthy fish, these enzymes mainly exist intracellularly, with highest activity in hepatocytes and minimal activity in serum. However, hepatopancreatic damage causes substantial release of GOT and GPT into blood, elevating serum activities. In this study, hepatopancreatic GOT and GPT activities increased initially then decreased with extract dosage, while serum activities showed the opposite trend, indicating that flavonoids in Caulis spatholobi ethanol extract reduce serum transaminase activities and protect liver function. Chen et al. found that Caulis spatholobi ethanol extract inhibited CCl -induced elevation of serum GOT and GPT activities. Kang et al. reported that total flavonoids from Caulis spatholobi improved alcohol-induced liver injury in mice by reducing serum GOT and GPT activities. These findings support our results.

C3, ALB, and IgM are important non-specific immune indicators in fish. C3 is the most abundant complement protein in serum, participating in various immune regulations including defense, regulation, and pathology. C3 must be activated to initiate subsequent complement cascade reactions. In this study, dietary supplementation with 0.32% Caulis spatholobi ethanol extract (T5) effectively increased serum C3 content, with the most significant difference from control. Peng et al. suggested that increased serum C3 content in grass carp may result from upregulated C3 mRNA expression, requiring further molecular investigation. Serum ALB functions in binding and transporting endogenous and exogenous substances, maintaining colloid osmotic pressure, scavenging free radicals, inhibiting platelet aggregation, and anticoagulation. In this study, 0.32% supplementation (T5) yielded the highest serum ALB content, indicating that Caulis spatholobi ethanol extract can increase ALB content, maintain osmotic pressure balance, promote substance exchange between blood and tissues, and enhance non-specific immunity. Among fish immunoglobulins, IgM is most abundant and plays a primary role in non-specific immunity. The lack of significant effect on serum IgM content in this study suggests that pathogens may be eliminated before entering the bloodstream, though the exact mechanism requires further investigation.

In the challenge test, cumulative mortality decreased initially then increased

with extract dosage, while immune protection rate showed the opposite trend, demonstrating that long-term feeding with Caulis spatholobi ethanol extract-supplemented diets can enhance non-specific immunity, improve survival, and prevent disease in yellow catfish.

Conclusion

1. Caulis spatholobi ethanol extract effectively enhanced SOD, CAT, and GSH-Px activities and GSH content while reducing MDA content in yellow catfish. It regulated NO content, decreased serum GOT and GPT activities, and increased C3 and ALB contents. Supplementation levels of 0.16% and 0.32% showed optimal effects on antioxidant capacity and non-specific immunity.
2. Dietary supplementation with 0.16% and 0.32% Caulis spatholobi ethanol extract effectively reduced cumulative mortality after bacterial challenge and improved immune protection rate.
3. Based on comprehensive evaluation of antioxidant capacity, non-specific immunity, and disease resistance, the optimal dietary inclusion level of Caulis spatholobi ethanol extract for yellow catfish is 0.16%–0.32%.

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Note: Figure translations are in progress. See original paper for figures.