

Utilization of mandarin orange peel waste as source of bioactive compounds in stripped catfish feed formulation

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Abstract. Susanto A, Agustina, Sukarti K, Agriandini M, Nurhalisa. 2026. Utilization of mandarin orange peel waste as source of bioactive compounds in stripped catfish feed formulation. *Asian J Agric* 10 (1): g100135. <https://doi.org/10.13057/asianjagric/g100135>. Mandarin orange peel contains bioactive compounds that can improve the health and growth of fish. The objective of this study is to analyze the bioactive compounds of mandarin orange peel to improve the health and growth of stripped catfish. Identification and determination of components of mandarin orange peel methanol extract used the LC-HRMS method. The study consisted of four treatments of orange peel powder with doses of 0, 2.5, 5.0, and 7.5 g kg⁻¹ feed. In total, 180 fish weighing 9.08±0.31 g in groups of 15 fish were kept in plastic containers for 30 days and fed twice a day (to satiation) according to the treatments. The observed parameters were hematology (erythrocytes, hemoglobin, hematocrit, and leukocyte differential), growth performance, feed utilization, and survival rate. Results of LC-HRMS showed 1.285 bioactive compounds detected in orange peel, with the highest levels for Herniarin 9.962490% and D-Glucuronic Acid 9.935390%. Mandarin orange peel powder in the feed affected hematological parameters, growth performance, and feed utilization in stripped catfish (P<0.05). Hemoglobin, leukocyte, monocyte, and neutrophil parameters were higher in the treatment group compared to the control group. The findings revealed that 2.5 g kg⁻¹ was the optimal dose because it produced the highest body weight, growth length, specific growth rate, feed utilization efficiency, and protein conversion ratio, with values of 6.69 g, 12.57 cm, 1.79% per day, 74.67%, and 2.02%, respectively (P<0.05). The survival rate for all treatments reached 100%. This approach supports mandarin orange peel as a biological resource containing bioactive compounds, thus supporting its use as a sustainable feed additive for stripped catfish.

Keywords: Bioactive, fish health, growth, mandarin orange peel, stripped catfish

INTRODUCTION

Stripped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878), is a species of freshwater fish native to Thailand. This fish is highly sought after for its delicious meat and high protein content. Stripped catfish farming has been carried out by the people and the fish is a desirable freshwater aquaculture commodity. This has led to an increase in stripped catfish farming, which in turn is in line with the increasing demand for feed for stripped catfish farming activities. The situation has resulted in high production costs, most of which come from feed, as it is still dependent on imported raw materials such as fish meal (Inez et al. 2024; Simarmata et al. 2024). The cost of meeting feed requirements during fish farming, especially intensive farming, can reach 70% of total production costs (Kari et al. 2022). This becomes one of the obstacles faced by fish farmers.

Feed additives are supplementary ingredients in feed that are useful for improving fish growth and health. The use of feed additives in fish farming is an alternative for producing good quality feed by utilizing cheaper and more readily available raw materials. Organic waste in the form

of fruit peels shows potential as an additive feed for fish, including those of apple (Qiang et al. 2019), banana (Susanto and Agustina 2023; Agustina et al. 2024) and dragon fruit (Xuan et al. 2024). The effectiveness of these fruit peels in improving fish growth and health is demonstrated by increased feed digestion efficiency and stimulation of the immune system.

Mandarin oranges, *Citrus reticulata* Blanco, are a type of widely cultivated sweet oranges. According to El-Ghfar et al. (2016), orange peel accounts for approximately 50-65% of the fruit's weight and is an affordable source of natural antioxidants. The peel of sweet oranges is rich in bioactive compounds such as pectin, phenols, alkaloids, tannins, and flavonoids; these active compounds function to enhance growth and immunostimulation (Doan et al. 2018). The protein content in mandarin orange peel ranges from 4.17-7.90%, while the fat content ranges from 7.73-13.58% (Yun and Liu 2024). In tilapia, adding mandarin orange peel powder at a dose of 15-20% in feed can increase amylase and protease enzyme activity (Reda et al. 2024). Results of research by Attalla et al. (2021) showed that a mixture of orange peel oil extract and powder at 2% in feed can improve growth, feed utilization, and immune

response in tilapia *Oreochromis niloticus* (Linnaeus, 1758). Meanwhile, Fadda et al. (2021) recommended using dried orange peel powder in tilapia feed to achieve optimal fish performance and save approximately 30% of yellow corn costs. Growth performance, feed utilization, oxidative stress resistance, and hematological parameters of rohu fish *Labeo rohita* (Hamilton, 1822) improved with 30% orange peel supplementation in the feed (Virk et al. 2023).

The purpose of bioactive analysis of mandarin orange peel using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) is to identify research gaps, namely the unexplored bioactives of orange peel so that it can describe and know the next research that will be carried out in the future in the application of mandarin orange peel as a source of sustainable feed additives. The use of advanced analytical techniques, such as LC-HRMS, facilitates the detection and quantification of precise bioactive compounds in mandarin orange peel, due to its high sensitivity (Wasito et al. 2022; Pudota et al. 2025).

Although studies on other species exist, the bioactive profile of mandarin peel has not been explored through LC-HRMS. Furthermore, this study utilized a traditional resource, mandarin peel, which is an agricultural byproduct and waste product, underutilized by humans. This is the novelty and significance of this study. These findings contribute to enhancing the value of mandarin peel as a fish feed additive, increasing stripped catfish production by improving feed quality, and reducing organic waste (mandarin peel) entering the environment.

MATERIALS AND METHODS

Location of study

Production of orange peel powder, fish maintenance, and LC-HRMS analysis were conducted at the Fish Nutrition Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. This research was conducted from September to October 2025.

Preparation of fish and container

Stripped catfish fry was obtained from a community nursery in the city of Samarinda in healthy condition. The stripped fry was acclimated in a circular plastic tank of a diameter of 1 meter filled with previously settled and aerated water. Adaptation lasted for 5 days, and the fish were fed commercial feed until satiated, twice daily at 7:00 AM and 5:00 PM. The rearing tanks were 150×100×50 cm fiber tanks filled with 40 L of water in a recirculating system. The water used in the tanks was allowed to settle for approximately 5 days, and each tank was aerated.

Production of mandarin orange peel powder and test feed

Mandarin orange peel powder was made from mandarin orange peels purchased in dried condition. The orange

peels were ground into a powder and mixed into the feed according to the treatments. The feed formulation is listed in Table 1. The mixture of feed ingredients and orange peel powder was then shaped using a feed and pellet-molding machine; the pellets were then dried in an oven at 50°C for approximately 3 hours. The dried feed was then stored in a closed plastic container. Proximate analysis was carried out to determine the levels of protein, lipid, ash, fiber, carbohydrate, C/P, and moisture according to the standard protocol established by the AOAC method (AOAC 2005).

Extraction of mandarin orange peel

Fresh fruit was thoroughly washed under running water, peeled manually, and cut into pieces using stainless steel scissors. The orange peels were then oven-dried at 50°C for 48 hours on aluminum foil. Once dry, the orange peels were ground using a grinder into a fine powder. The powdered orange peels were then extracted according to Gómez-Mejía et al. (2023). The mandarin orange peel powder was macerated in methanol solvent, with a weight-to-volume ratio of 1:10. This process followed the sequential cold maceration method described by Kusumawati et al. (2025), resulting in a methanol extract fraction that was then analyzed to determine its bioactive compounds.

Table 1. Nutritional content of experimental feeds containing mandarin orange peel powder

Material (100g/kg feed)	Treatment			
	T0	T1	T2	T3
Fish meal	32.00	32.00	32.00	32.00
Soybean flour	25.70	25.70	25.70	25.70
Wheat flour	14.50	14.50	14.50	14.50
Bran flour	10.00	10.00	10.00	10.00
Fish oil	2.00	2.00	2.00	2.00
Corn oil	2.00	2.00	2.00	2.00
Vitamin and mineral mix	6.00	6.00	6.00	6.00
Mandarin orange peel powder	0.00	0.25	0.50	0.75
Choline chloride	2.00	2.00	2.00	2.00
Carboxymethyl cellulose	2.00	2.00	2.00	2.00
Organic chromium	0.18	0.18	0.18	0.18
Cellulose	3.62	3.37	3.57	2.87
Proximate analysis (%)				
Moisture	4.91	5.82	5.46	5.05
Ash	16.07	14.76	15.28	17.34
Crude protein	32.79	33.79	34.33	35.43
Crude fats	4.68	4.26	4.20	5.26
Crude fiber	5.75	5.87	5.26	4.20
Carbohydrates	35.80	35.50	35.07	32.73
Energy (Kcal)	242.17	241.52	241.85	248.44
C/P	7.39	7.15	7.04	7.01

Note: T0: 0, T1: 2.5, T2: 5.0, T3: 7.5 g orange peel powder per kg of feed. This feed formulation is based on calculations for 100 g/kg feed

Bioactive analysis using LC-HRMS

Identification and determination of components in the methanol extract of mandarin orange peel were carried out using the LC-HRMS method with a Thermo Scientific™ Vanquish™ UHPLC Binary Pump Liquid Chromatography instrument and a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ High Resolution Mass Spectrometer. A 500 mg sample of the methanol extract fraction of *C. reticulata* mandarin orange peel powder was diluted in 1 mL of methanol, vortexed for 2 minutes, and sonicated at 20°C for 15 minutes. After centrifugation at $3842 \times g$ for 5 minutes, the sample was filtered through a 0.2 µm nylon filter and prepared for injection. The mobile phase involved two eluents: Eluent A containing water and 0.1% formic acid, and Eluent B containing methanol + 0.1% formic acid. The analytical column contained phenyl hexyl 100 mm \times 2.1 mm ID \times 2.6 µm, and used a gradient technique with a flow rate of 0.3 mL/min and a sample injection volume of 3 µL. The mobile phase of sample B was set at 5%, gradually increased to 90% within 16 minutes, held at 90% for 4 minutes, and allowed to progress to the initial condition (5% B) for up to 25 minutes. For the sample preparation and examination procedure, \pm 0.1 mg of extract was dissolved in methanol for LC-MS, homogenized with a vortex mixer, and filtered with 0.2 µm mil.; 5 µL of the mixture was then injected into the HRMS, and the examination process was performed, after which the results were obtained. This procedure followed the method of Muin and Taufek (2024) and Kusumawati et al. (2025).

Experimental design

The study used a completely randomized laboratory experiment with a design consisting of four treatments and three replications. The treatments involved fish feed with various doses of mandarin orange peel powder: 0.0 g/kg (T0/control), 2.5 g/kg (T1), 5.0 g/kg (T2), and 7.5 g/kg (T3). The adapted stripped catfish, with an initial weight of 9.08 ± 0.31 g, were then placed in rearing tanks, each with 15 fish. The fish were reared for 30 days and given the treatment feed twice daily at 7:00 AM and 5:00 PM. During rearing, siphoning was performed every two days, with new water added equal to the amount of water removed during siphoning. The treatments and repetitions referred to Agustina et al. (2024) and Agustina et al. (2025). The selection of the dose refers Attalla et al. (2021), to the fact that 2% orange peel flour in feed can increase growth, feed utilization, and immune response in tilapia.

Parameter observed

The following were the observed parameters in this study:

Growth (g) = final weight - initial weight (Nurhalisa et al. 2022; Muin and Taufek 2024)

Specific growth (% day⁻¹) = $[\text{Ln}(\text{final weight}) - \text{Ln}(\text{initial weight})]/t \times 100\%$ (Muin and Taufek 2024)

Feed utilization efficiency (%) = $[(\text{final weight} + \text{dead weight}) - \text{initial weight}]/\text{feed consumption} \times 100\%$ (Aisyah et al. 2021)

Protein efficiency ratio (%) = $\text{Fish weight gain} / \text{Protein intake}$ (Salem and Abdel-Ghany 2018)

Survival (%) = $(\text{Final number of fish} / \text{Initial number of fish}) \times 100\%$ (Salem and Abdel-Ghany 2018)

Mortality is determined by observing the number of fish that die (Nurhalisa et al. 2022).

The Relative Survival Percentage (RPS) value was obtained by comparing the number of fish deaths in the treatment and control groups (Nurhalisa et al. 2022).

The number of lactic acid bacteria in the catfish intestines was also observed at the beginning and end of rearing, referring to the pour plate method (Mousavi et al. 2019), with modifications using MRSA media.

Before blood collection, the fish were anesthetized by immersing them in ice at 4°C to prevent stress and pain prior to death. The observed hematological parameters in this study were hemoglobin levels, hematocrit, total erythrocytes and leukocytes, and leukocyte differentials (lymphocytes, monocytes, and neutrophils). These parameters were observed at the beginning and end of fish maintenance (days 0 and 30). For the process, blood samples were taken from the caudal vein using a syringe. The Sahli method with a hemometer was used to assess hemoglobin levels. The hematocrit level referred to Nurhalisa et al. (2022), the leukocyte differential referred to Andayani et al. (2023), and the number of erythrocytes and leukocytes in the fish referred to Nurhalisa et al. (2022).

The measured water quality parameter was DO, using a Horiba U-10. Total ammonia was measured using a UV-Vis Spectrophotometer, with brand B-ONE and model UV-Vis 100 DA-X.

Data analysis

LC-HRMS data in the form of chromatograms and sample spectra were processed using Compound Discovery 3.0 software. Compound Discovery 3.0 data processing consisted of several stages: sample chromatogram creation, noise reduction, identification based on molecular weight, and data compilation. For data translation, the read compound data was adjusted by a method using compound references from MzCloud Mass, ChemSpider, and PubChem, in accordance with the method of Tuldjanah et al. (2024). Growth, survival, and hematological data were statistically analyzed by One-Way ANOVA followed by Duncan's multiple range test using SPSS 12.0. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Bioactive content identification of mandarin orange peel powder

Identification of bioactive compounds in the methanol extract of mandarin orange peel powder using the LC-HRMS method resulted in 1,285 detected compounds (Figure 1). Among the 20 bioactive compounds with the highest concentrations (Table 2) were coumarins (such as Herniarin), organic acids (such as D-Glucuronic acid), and fatty acids (such as Corchorifatty acid F). Representative mass spectra for key compounds are shown in Figure 2.

The dominant compounds (Table 2) in mandarin peel are coumarins (such as Herniarin), organic acids (such as D-Glucuronic acid), and fatty acids (such as Corchorifatylic acid F). These three dominant bioactive compounds play a role in enhancing physiological responses to growth, survival, and hematology.

Growth, feed utilization, and survival rate of stripped catfish

Dietary inclusion of mandarin orange peel powder significantly affected ($P < 0.05$) all growth and feed utilization parameters except survival, mortality, and RPS (Table 3). The T1 treatment (2.5 g/kg) yielded the highest weight gain, specific growth rate, and feed efficiency, which were significantly superior to the control (T0) and other treatment groups (T2, T3).

The number of lactic acid bacteria

The addition of mandarin orange peel powder to the feed significantly affected ($P < 0.05$) the number of lactic

acid bacteria (Table 4). Treatment T1 (2.5 g/kg) resulted in the highest increase in the number of lactic acid bacteria in the intestines of the catfish compared to the control (T0) and other treatment groups (T2, T3).

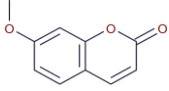
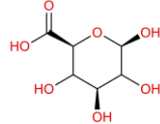
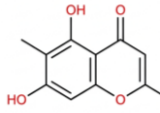
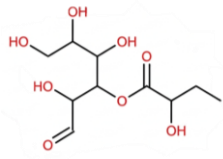
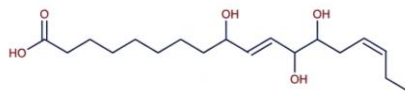
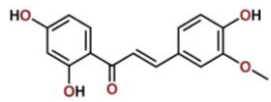
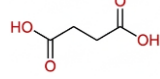
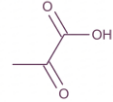
Hematology of stripped catfish

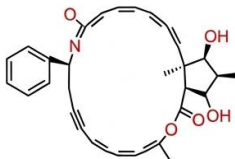
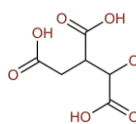
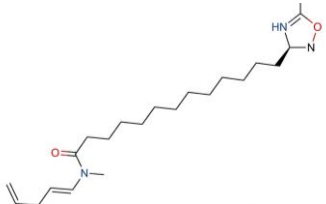
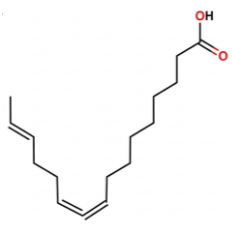
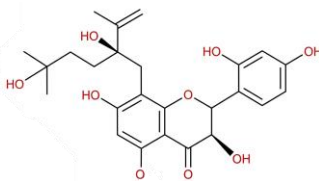
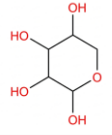
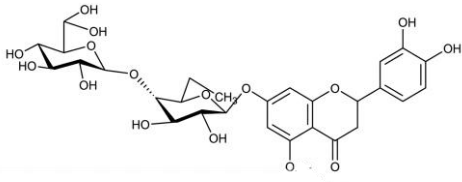
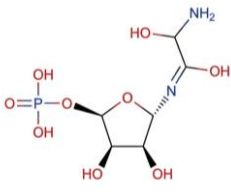
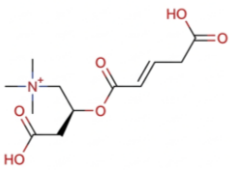
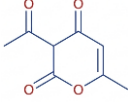
The addition of mandarin orange peel powder to the feed significantly affected ($P < 0.05$) all hematological parameters (Table 5). The T1 treatment (2.5 g/kg) had the highest hematocrit, erythrocyte, and neutrophil levels, which were significantly superior to the control (T0) and other treatment groups (T3, T2).

Water quality

Water quality parameters during the study were optimal and did not affect the research results (Table 6).

Table 2. Results of identification and determination of the methanol extract content of mandarin orange peel flour (*Citrus reticulata*)

No	Compound name	Chemical Structure Image	Formula	Content (%)
1.	Herniarin		$C_{10}H_8O_3$	9.962490
2.	D-Glucuronic acid		$C_6H_{10}O_7$	9.935390
3.	Eugenitol		$C_{11}H_{10}O_4$	9.077460
4.	Glucose 3-hydroxybutyrate		$C_{10}H_{18}O_8$	8.924680
5.	Corchorifatty acid F		$C_{18}H_{32}O_5$	8.839170
6.	Homobutein		$C_{16}H_{14}O_5$	8.681900
7.	Succinic acid		$C_4H_6O_4$	8.481280
8.	Pyruvic acid		$C_3H_4O_3$	8.277570

9.	Viridenomycin		$C_{34}H_{37}NO_6$	7.913120
10.	isocitric acid		$C_6H_8O_7$	7.714090
11.	Laucysteinamide A		$C_{23}H_{38}N_2OS$	7.295430
12.	Linoleic acid		$C_{18}H_{32}O_2$	6.699850
13.	Kushenol H		$C_{26}H_{32}O_8$	6.523580
14.	Xylose		$C_5H_{10}O_5$	6.293890
15.	5-O-Methylerythriodictyol glucosyl- (1->4) -galactoside	7- 	$C_{28}H_{34}O_{16}$	5.604850
16.	Glycineamide ribotide		$C_7H_{15}N_2 O_8P$	5.541990
17.	Glutacony L-carnitine		$C_{12}H_{19}N O_6$	4.445030
18.	Dehydroacetic acid		$C_8H_8O_4$	4.410430

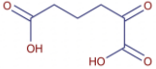
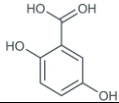
19.	Oxoadipic acid		C ₆ H ₈ O ₅	3.718260
20.	Gentisic acid		C ₇ H ₆ O ₄	3.392210

Table 3. Growth performance, feed utilization, and survival rate of stripped catfish fed diets containing mandarin orange peel flour

Parameter	Treatment				p-value ANOVA
	T0	T1	T2	T3	
Initial weight (g)	8.74±0.40 ^a	9.08±0.71 ^b	8.99±0.56 ^a	9.49±0.3 ^a	0.050
Final weight (g)	12.68±0.61 ^a	16.02±2.33 ^b	13.90±2.18 ^a	15.72±0.12 ^a	0.015
Weight gain (g)	4.18±0.75 ^a	6.69±1.74 ^b	4.16±0.79 ^a	4.23±0.36 ^a	0.020
Initial length (cm)	10.51±0.33 ^a	10.66±0.43 ^b	10.93±0.65 ^a	11.34±0.18 ^a	0.032
Final length (cm)	11.93±0.44 ^a	12.57±0.55 ^b	12.32±1.12 ^a	12.56±0.32 ^a	0.050
Length growth (cm)	1.67±0.50 ^a	1.91±0.15 ^b	1.39±0.50 ^a	1.22±0.18 ^a	0.041
Specific growth rate (% day ⁻¹)	1.24±0.25 ^a	1.79±0.29 ^b	1.18±0.07 ^a	1.05±0.10 ^a	0.045
Feed consumption rate (g)	135.40±4.02 ^a	132.98±14.83 ^b	143.75±5.39 ^a	133.95±2.95 ^a	0.050
Feed utilization efficiency (%)	46.47±9.01 ^a	74.67±11.36 ^b	43.32±7.10 ^a	47.31±3.10 ^a	0.025
Protein efficiency ratio (%)	1.24±0.25 ^a	2.02±0.31 ^b	1.11±0.18 ^a	1.21±0.08 ^a	0.050
Mortality (%)	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.070
Survival rate (%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	0.062
RPS (%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	0.060

Note: T0: 0, T1: 2.5, T2: 5.0, T3: 7.5 g orange peel flour kg⁻¹ ration. The ANOVA p-value is significant (P<0.05). The statistical effect size ($\alpha=0.05$) and 95% confidence interval. Different letters on each line indicate different groups

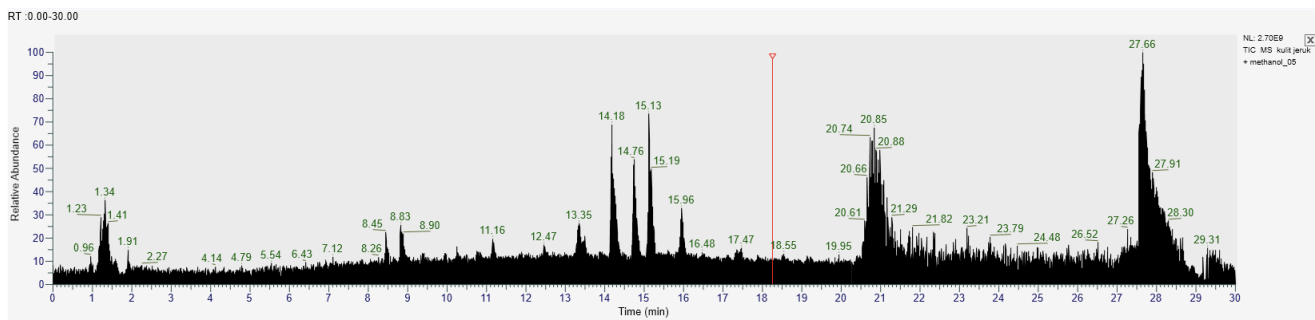
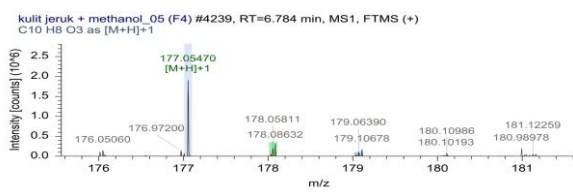
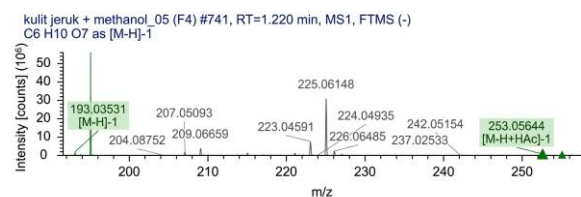


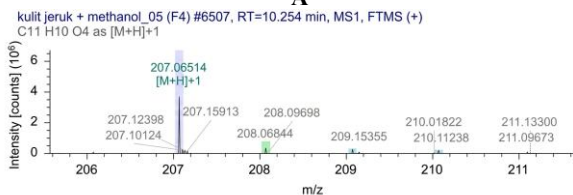
Figure 1. Chromatogram of methanol extract of mandarin orange peel flour (*Citrus reticulata*) using LC-HRMS



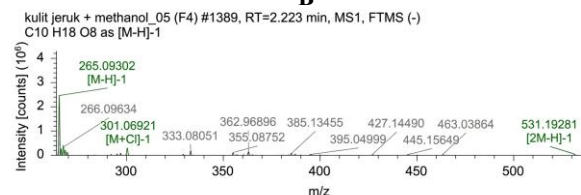
A



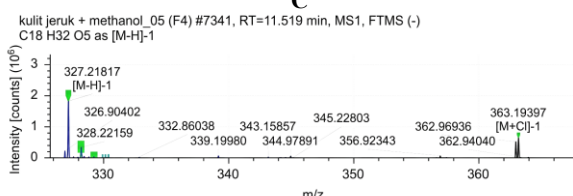
B



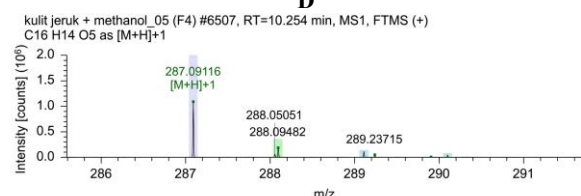
C



D



E



F

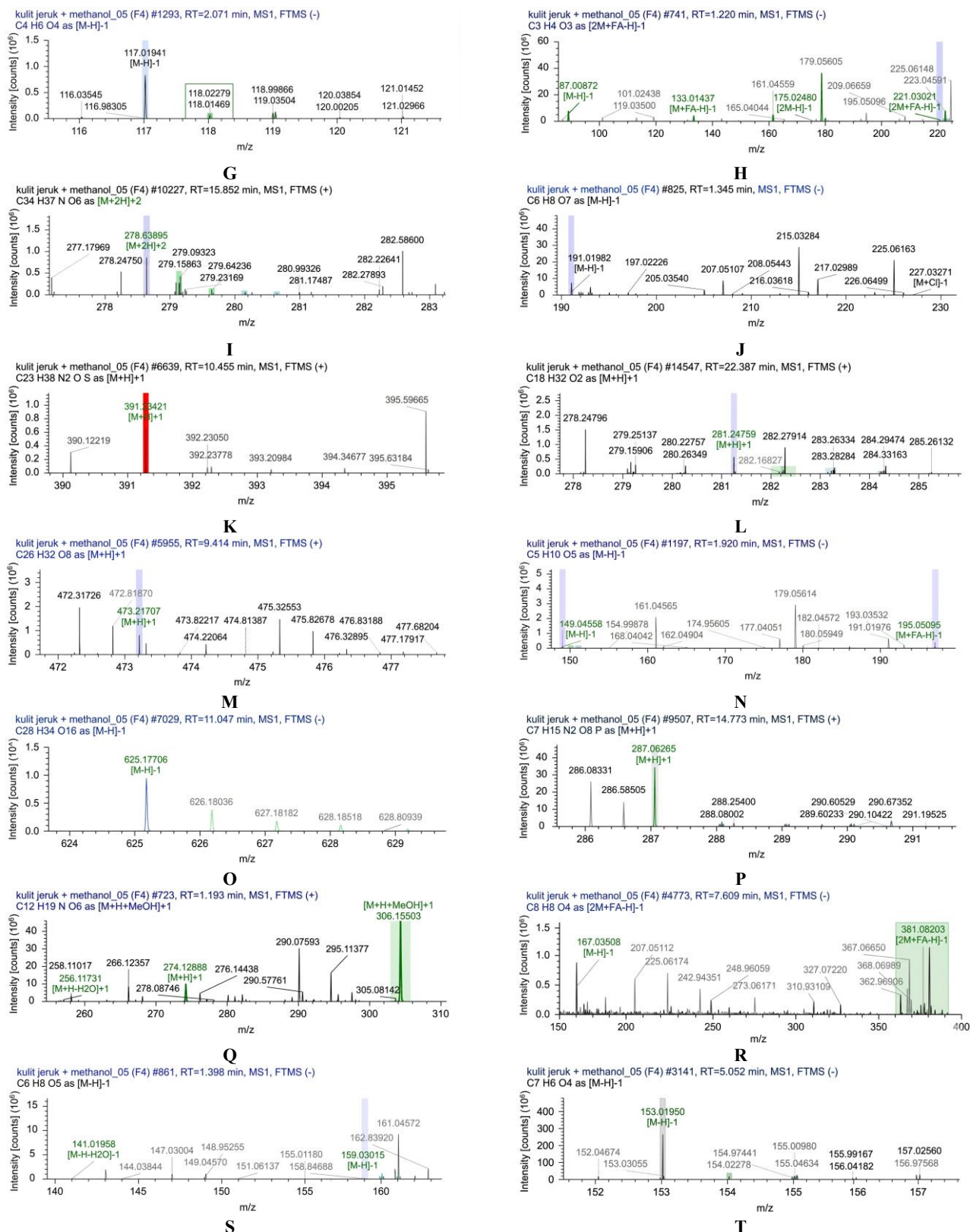


Figure 2. Mass spectrometry results of compounds. A. Herniarin, B. D-Glucuronic acid, C. Eugenitol, D. Glucose 3-hydroxybutyrate, E. Corchorifatty acid F, F. Homobutein, G. Succinic acid, H. Pyruvic acid, I. Viridenomycin, J. Isocitric acid, K. Laucysteinamide A, L. Linoleic acid, M. Kushenol H, N. Xylose, O. 5-O-Methylerythrodiol 7-glycosyl- (1->4) -galactoside, P. Glycineamide ribotide, Q. Glutacony L-carnitine, R. Dehydroacetic acid, S. Oxoadipic acid, T. Gentisic acid

Table 4. The number of lactic acid bacteria in the intestines of stripped catfish during observation

Parameter	Day	Treatment				p-value ANOVA
		T0	T1	T2	T3	
The number of lactic acid bacteria (log CFU mL ⁻¹)	0	3.36±0.99 ^a	3.40±0.44 ^b	3.42±0.67 ^a	3.50±0.60 ^a	0.043
	30	3.22±0.04 ^a	3.95±0.23 ^b	3.68±2.55 ^a	3.20±1.65 ^a	0.042

Note: T0: 0, T1: 2.5, T2: 5.0, T3: 7.5 g orange peel flour kg⁻¹ ration. The ANOVA p-value is significant (P<0.05). The statistical effect size ($\alpha=0.05$) and 95% confidence interval. Different letters on each line indicate different groups

Table 5. Hematology of stripped catfish fed diets containing mandarin orange peel flour

Parameter	Day	Treatment				p-value ANOVA
		T0	T1	T2	T3	
Hemoglobin (g dL ⁻¹)	0	4.70±0.99 ^a	4.70±0.99 ^a	4.70±0.99 ^a	4.70±0.99 ^a	0.063
	30	5.87±0.04 ^a	9.00±0.23 ^a	7.47±2.55 ^a	12.47±1.65 ^b	0.042
Hematocrit (%)	0	18.50±4.95 ^a	18.50±4.95 ^a	18.50±4.95 ^a	18.50±4.95 ^a	0.052
	30	15.33±1.02 ^a	26.67±0.69 ^b	17.67±3.36 ^a	18.33±4.67 ^a	0.030
Erythrocytes (×10 ⁶ cell mm ³ ⁻¹)	0	1.25±0.42 ^a	1.25±0.42 ^a	1.25±0.42 ^a	1.25±0.42 ^a	0.055
	30	1.25±0.07 ^a	1.59±0.15 ^a	1.40±0.19 ^b	1.31±0.03 ^a	0.040
Leukocytes (×10 ⁴ cell mm ³ ⁻¹)	0	1.88±0.24 ^a	1.88±0.24 ^a	1.88±0.24 ^a	1.88±0.24 ^a	0.060
	30	1.90±0.49 ^a	1.97±0.19 ^a	1.96±0.33 ^a	2.86±0.72 ^b	0.032
Lymphocytes (%)	0	92.33±2.01 ^a	92.33±2.01 ^a	92.33±2.01 ^a	92.33±2.01 ^a	0.061
	30	93.56±1.88 ^a	92.00±0.97 ^a	91.73±1.67 ^a	91.94±1.11 ^b	0.053
Monocytes (%)	0	5.12±1.34 ^a	5.12±1.34 ^a	5.12±1.34 ^a	5.12±1.34 ^a	0.060
	30	4.53±1.48 ^a	4.90±0.96 ^a	5.43±1.22 ^a	5.87±0.91 ^a	0.055
Neutrophils (%)	0	2.56±0.67 ^a	2.56±0.67 ^a	2.56±0.67 ^a	2.56±0.67 ^a	0.061
	30	1.90±1.41 ^a	3.11±0.18 ^a	2.84±0.46 ^a	2.19±1.48 ^b	0.033

Note: T0: 0, T1: 2.5, T2: 5.0, T3: 7.5 g orange peel flour kg⁻¹ ration. The ANOVA P-value is significant (P<0.05). The statistical effect size ($\alpha = 0.05$) and 95% confidence interval. Different letters on each line indicate different groups

Table 6. Water quality parameters during stripped catfish maintenance

Water quality	Measurement results	Reference
pH	6.9-7.0	6.9-7.3 (Agustina et al. 2024)
DO (ppm)	5.0-5.2	4.75-5.20 (Agustina et al. 2024)
Temperatur (°C)	27.6-27.8	25-31 (Wardani et al. 2021)
Total ammonia nitrogen (ppm)	0-0.01	0-0.01 (Wardani et al. 2021)

Discussion

A low dose (2.5 g/kg) of mandarin orange peel powder optimally increased growth, feed utilization and health in stripped catfish fry. The level of stripped catfish feed utilization with the addition of mandarin orange peel powder in the feed at a dose of 2.5 g kg⁻¹ was higher than the other treatments (Table 3). This dosage is optimal for optimal growth. In other words, this dosage is effective in supporting feed utilization and growth of stripped catfish. This aligns with research by Salem and Abdel-Ghany (2018) found that a 2% dose of orange peel powder in tilapia feed could improve digestion and absorption of nutrients. Meanwhile, Fadda et al. (2021) stated that the best dose of mandarin orange peel powder in increasing tilapia feed utilization for is 20%. When compared to the stripped catfish in this study, tilapia required less mandarin orange peel powder to increase feed utilization and growth.

According to the results of this study, stripped catfish have a lower tolerance or optimal dose, being 2.5 g/kg of feed. This is because stripped catfish are omnivorous but tending to be carnivorous; thus, their digestive system's ability to utilize fiber is limited. In comparison, tilapia

showed the best growth performance with a dose of 2% lemon peel, and Ningu fish (*Labeo victorinus* (Boulenger, 1901)) showed the best growth performance after given feed with more than 5% lemon peel (Ngugi et al. 2017; Toutou et al. 2018).

Evaluation of the Relative Survival Percentage (RSS) parameter during the observation period showed very positive results, where all treatment doses applied successfully recorded the best survival rates. This optimal survival indicator is concrete evidence that the treatment of adding mandarin orange peel flour to the feed is well tolerated by the fish's digestive system, does not contain lethal substances, and is guaranteed safe because it has been proven not to cause a risk of death (Nurhalisa et al. 2022).

There was an increase in the number of lactic acid bacteria in the fish intestines at the beginning and after treatment. Treatment with 2.5 g/kg of mandarin orange peel powder resulted in the highest number of lactic acid bacteria, which was 3.95 log CFU mL⁻¹. Lactic acid bacteria are beneficial microorganisms whose main function is to balance the intestinal microflora and

maximize the absorption process of various important nutrients, which will ultimately contribute greatly to accelerating growth and maintaining the overall health of fish (Agustina et al. 2025).

The addition of mandarin orange peel powders up to a dose of 7.5 g kg⁻¹ of feed did not cause health problems in stripped catfish. This is indicated by hematological parameter values, which were generally within the normal range. The addition of mandarin orange peel powder improved the health of stripped catfish (increased Hb, hematocrit, erythrocytes, leukocytes, lymphocytes, monocytes and neutrophils). This was due to the presence of bioactive compounds in mandarin orange peel (LC-HRMS results, Table 1), such as Herniarin (phenol) and Homobutein and Kushenol H (flavonoids). However, further research is needed regarding the specific effects of these bioactive compounds on fish.

The 20 bioactive compounds with the highest concentrations in mandarin orange peel in Table 2 can be classified according to their chemical groups. This study presents the novelty of mandarin peel profiling based on LC-HRMS, as it provides a comprehensive chemical basis, by identifying specific compounds such as Herniarin and D-Glucuronic Acid (Table 2) that need further investigation in aquatic models. This study presents possibilities for future research to be carried out. Possible research may involve deeper analysis of fish health parameters, growth performance, feed utilization, and survival in relation to one of the bioactive compounds in mandarin orange peel mentioned in Table 3, which need further investigation in aquatic models. In relation to future ethnobotany or applied aquaculture research, namely the utilization of agricultural products due to their bioactive compound content can be used for fish feed additives in aquaculture to support the health, growth and survival of fish, thereby reducing dependence on chemical drugs or antibiotics.

In conclusion, the addition of 2.5 g kg⁻¹ mandarin orange peel powder to fish feed was found to be the optimal dose, as it improved the health of stripped catfish as analyzed through hematology, growth performance, feed utilization, and survival rate. Thus, the utilization of mandarin orange peel in this study emphasizes the sustainable use of biological resources, reduction of waste and antibiotic dependence, and potential socio-ecological relevance. More specifically and in-depth, the use of mandarin orange peel has proven to have great potential for application as a highly effective fish feed additive, while also being an alternative source of functional nutrients that are environmentally friendly and sustainable. The long-term prospects of utilizing this natural ingredient are expected to significantly reduce, or even replace, the high level of farmer dependence on the use of synthetic antibiotics in intensive catfish farming practices. Although this study has successfully identified the presence of various potentially beneficial bioactive compounds, a limitation of this study is that it has not yet been isolated or individually tested for each compound to determine its absolute contribution to the observed positive effects. Therefore, further investigation is urgently needed to

elucidate the fundamental biological mechanisms underlying the immunostimulation process and increased growth rate, particularly when viewed from molecular aspects such as the regulation of gene expression levels and the dynamics of changes in microbiota composition in the fish digestive tract.

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