Impact of Dietary Nutmeg (*Monodora myristica*) Seed Extract on Growth Performance, Immune Response, Gut Morphology, Liver Indices, and Hematological Parameters in African Catfish (*Clarias gariepinus*)

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ABSTRACT

The use of synthetic chemicals as growth promoters and immune boosters in intensive aquaculture has significant negative impacts on the aquatic environment and poses potential risks to human health, necessitating the need for organic alternatives. This study investigated the effects of ethanol extract from *Monodora myristica* seed (EMM) as a growth promoter, immune booster, and gut area modulator in African catfish (*Clarias gariepinus*). The crude extract was obtained using a conventional method from finely ground, dried seeds of *M. myristica*. The extract was incorporated into a 40% crude protein diet at inclusion levels of 0.00% (M0), 0.05% (M1), 0.10% (M2), 0.15% (M3), and 0.20% (M4). These five diets were randomly assigned to triplicate groups of *C. gariepinus* (2.32± 0.03 g), housed in 15 plastic aquaria for 84 days. Results showed that growth performance and feed conversion ratio were improved in the M2 group. Fish fed EMM diets also exhibited increased gut absorption area. Changes in the haematological indices, serum biochemistry, and liver oxidative biomarkers indicated enhanced anti-oxidant and immune responses with EMM inclusion. Specifically, total protein, glutathione peroxidase, glutathione, and glutathione S-transferase levels significantly increased ($p<0.05$). Survival rates were higher in M2 and M3 (91.00%) than in the control group (86.67%). This study highlights the positive effects of EMM inclusion at a moderate concentration (0.10%) in the diet of *C. gariepinus*, particularly on immune response and survival rate.

Keywords: Catfish, Immunocompetence, Oxidative stress, Phytogenic

INTRODUCTION

The growth in aquaculture production recorded in Africa is driven by the increased culture capacity established in the recent years, as well as national policies fueled by rising local demand for animal protein. Despite this development, overall aquaculture production in Africa is expected to reach slightly above 3.2 million tonnes in 2030, with Egypt producing the majority (2.2 million tonnes) (FAO, 2020). According to FAO (2020), this modest contribution from the continent will likely decline due to factors such as inadequate water availability, limited production locations, increased adoption and enforcement of environmental protection regulations, and most importantly, the susceptibility of cultured fish species to diseases resulting from stress associated with intensification.

High stocking density, build-up of nitrogen compounds from uneaten feeds, and oxidative stressors are common features of intensification, leading to poor performance, weakened immune system, and increased susceptibility to infectious

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diseases. These factors result in significant financial losses for farmers (Abdelsalam *et al*., 2017). To ensure optimal production, various synthetic chemicals and antibiotics have traditionally been used as growth promoters and antioxidants. However, concerns about the use of antibiotics in aquaculture-including their potential to accelerate antibiotic resistance in bacteria, human health risks, ecological disruption, and habitat destruction-have discouraged their use (Ajani *et al*., 2020). Recently, herbs, plant extracts, and essential oils have received great attention as feed additives in fish diets to improve growth and increase production. The efficacy of plant-based ingredients as growth promoters and immune boosters in fish has been demonstrated for *Ociumum gratissimum* (Abdel-Tawwab *et al*., 2018), *Psidium guajava* (Omitoyin *et al*., 2019), and *Rosemarinus officinalis* (Yousefi *et al*., 2019).

The tropical tree, *Monodora myristica* (calabash nutmeg), belongs to the custard apple family (Annonaceae) of flowering plants. It is a berry native to West Africa's evergreen woodlands and contains anti-inflammatory, antioxidant, antibacterial, and antifungal bioactive compounds (Agiriga and Siwela, 2017). The seed extract contains major lipids and pharmacologically active components such as alkaloids, flavonoids, and vitamins A and E. According to Seneme *et al*. (2021), the predominant active ingredient in the plant is myristicin (1-allyl-5-methoxy-3,4-methylenedioxybenzene). Fournier *et al*. (1999) found that the essential oil from the seeds contains pinene, phellandrene, limonene, and myrcene, which enhance the activity of digestive enzymes. Although Sodamola *et al*. (2017) found that *C. gariepinus* given 2.5 percent *M. myristica* powder exhibited improved nutrient utilization, little information is available on the use of extracts from this plant as a growth promoter and antioxidant.

Ethanol is generally recognized as a safe solvent and is therefore widely used in antioxidant extraction (Mariana *et al*., 2013). Although bioactive substances in plant material are typically not extracted using a single solvent, binary solvent mixtures, such as water and an organic solvent, are often preferred (Lim *et al*., 2019). For example, binary ethanol extracts of scent leaf (*Occimum gratissimum*) are reported to be more effective than the powdered form of the leaf (Ukorojie *et al*., 2018). Similarly, ethanol extracts of *Phyllanthus niruri, Curcuma longa, Zingiber officinale, Citrus limon,* and *Cymbopogon citratus* have shown higher phenolic content and antioxidant activity than the aqueous extracts (Atun *et al*., 2023).

The African catfish (*Clarias gariepinus*) is the most farmed fish species in Nigeria (Kaleem and Sabi, 2021) due to its numerous advantages, including rapid growth rates that allow for efficient production cycles and quicker returns on investment, successful captive reproduction ensuring a continuous supply of fingerlings, and resilience to various environmental conditions. Its taste, texture, and nutritional value have made African catfish a popular choice among consumers, driving demand for the species in the Nigerian market. Therefore, this study, investigated the effect of ethanol extract of *M. myristica* seeds on the growth, health status and gut absorption area of African catfish.

MATERIALS AND METHODS

Extraction of Monodora myristica seed and phytochemical screening

Dried seeds of *Monodora myristica* were procured from a local market in Ile-Ife, Nigeria. The seeds were broken to release the kernels and reduced to fine particles using an electric blender. One hundred grams of blended seeds were soaked in 400 mL of aqueous ethanol (70% v/v, 1,000 mL) in a sterile desiccator, stirred to mix, and left to stand for 48 h at room temperature. The filtrate was condensed and concentrated under reduced pressure in a rotary evaporator (Búchi-R11, Switzerland) at 150 rpm and 80 °C, resulting in a brown residue (Thomsone *et al*., 2012). The extract was screened for alkaloids, tannins, flavonoids, saponins and phlobatannins following the procedures of Trease and Evans (2002) using Dragendorff, Mayers and Hagners reagents.

Experimental feed production and feeding

A 40% crude protein diet was formulated, with ethanol extract of *M. myristica* (EMM) included at 0.00% (control), 0.05%, 0.10%, 0.15% and 0.20% (M0, M1, M2, M3, and M4, respectively), as shown in Table 1. All ingredients were reduced to fine particles using grinding mills, and weighed quantities were thoroughly mixed with test ingredients into a homogenous mass in separate plastic containers, with edible starch used as a binder. The mash was pelletized using a 2 mm die hand-driven pelletizer to form pellets, air-dried, and then packed into well-labelled air-tight plastic containers until use. Diets were prepared every three weeks. Proximate composition analysis was conducted following AOAC (2005) methods.

Experimental design and fish culture

After a 14-day acclimatization period, a total of 300 *Clarias gariepinus* fingerlings (2.32± 0.03 g) were randomly allocated to 15 experimental plastic aquaria (0.42×0.29×0.25 m each) at a density of 20 fish per tank. The five diets were each fed to three groups of fish at 3% body weight in two

Table 1. Composition of experimental diets with varying levels of *Monodora myristica* oil extract and their analyzed proximate compositions.

Ingredients (%)	**M0 (0%)**	**M1 (0.05%)**	**M2 (0.10%)**	**M3 (0.15%)**	**M4 (0.20%)**
Fishmeal	16.53	16.53	16.53	16.53	16.53
Ground nut cake	24.78	24.78	24.78	24.78	24.78
Soybean meal	33.17	33.17	33.17	33.17	33.17
Maize	10.01	10.01	10.01	10.01	10.01
Wheat offal	10.01	10.01	10.01	10.01	10.01
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Di-calcium phosphate	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Starch	1.50	1.45	1.40	1.35	1.30
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50
Premix	1.50	1.50	1.50	1.50	1.50
EMM	0.00	0.05	0.10	0.15	0.20
Total	100.00	100.00	100.00	100.00	100.00
Analyzed composition					
Protein	39.59	40.01	39.35	40.88	40.97
Ether extract	7.43	6.79	5.27	6.05	5.23
Fibre	2.63	2.45	1.65	1.83	2.14
Ash	9.33	9.26	8.80	9.45	8.81
Moisture	4.65	2.72	6.89	2.98	3.97

Note: Premix composition: Vitamin B12 = 15 mcg; B6 = 20,000 mg; Vitamin B5 = 40 mg; Vitamin B4 = 4,000 mcg; Vitamin B3 = 90,000 mg; Vitamin B1 = 2,000 mg; Vitamin B2 = 15,000 mg; Vitamin A = 2,050,000 IU; Vitamin B = 500 mcg; Vitamin C = 350,000 mg; Vitamin D3 = 4,250,000 IU; Vitamin E = 2,500.000 IU; Vitamin K = 8,000 mg;, Copper Sulphate = 4,000 mg; Inositol = 50,000 mcg; Potassium Iodine = 2,000 mg; Inositol = 50,000 mg; Methionine = 50,000 mg; Choline Chloride = 600,000 mg; Ferrous Sulphate = 40,000 mg; Manganese oxide = 30,000 mg; Magnesium = 60,000 mcg; Molybdenum = 100 mg; Antioxidant = 125,000 mg; Cobalt = 750 mg; Sodium Selenite = 200 mcg; Zinc oxide = 40,000 mg; EMM = ethanol extract of *M. myristica*

daily installments, between 7.00–8.00 a.m. and 4.00–5.00 p.m. Fish were weighed bi-weekly, and feed requirements were adjusted accordingly. Throughout the experiment, water quality parameters were maintained as follows: dissolved oxygen (DO) ranged from 4.6–4.9 mg·L^{-1}, pH ranged from 6.5–7.2, temperature ranged from 25.5–26.2 °C, and nitrite ranged 0.05–0.15 mg·L^{-1}. DO and temperature were measured using a YSI Combined Digital Probe (Model 57; VWR Company and New Jersey), pH using Photic 20 digital pH meter (Labtech International, Heathfield, UK), and nitrite using a commercial test kit.

Assessment of growth and survival of experimental fish

Growth and nutrient utilization parameters were determined according to Castell *et al.* (1980) using bi-weekly measurements over the 84–day feeding experiment. The following formula was used:

$$\text{Feed intake (g)} = \text{Sum of feed throughout the experimental period;}$$

$$\text{Weight gain (g)} = w_2\text{-}w_1$$

where w_1 is initial weight and w_2 is final weight;

$$\text{Specific Growth Rate} = \frac{\text{Loge } W_2\text{-Loge } W_1}{T_2\text{-}T_1} \times 100$$

where Log_e = Natural logarithm and T_2-T_1 is the total days of experiment from start, T_1 to finish, T_2;

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}};$$

$$\text{Protein efficiency Ratio (PER)} = \frac{\text{Mean weight gain}}{\text{Protein intake}}$$

where

Protein Intake = Feed Intake × % protein in diets ;

$$\text{Survival rate (\%)} = \frac{\text{Numbers of fish at } T_2}{\text{Numbers of fish at } T_1} \times 100.$$

Measurement of gut villi and estimation of area of absorption

Six fish per treatment were tranquilized using buffered tricaine methanesulfonate (MS-222) at 30 mg·L^{-1}, and the intestines were excised aseptically for histological study. Mid-guts were prepared on slides as described by Culling (1974) and Carleton *et al*. (1967). Length of villus (LV, µm), width of villus (WV, µm), and crypt depth (CD, µm) were measured in triplicates at 40X magnification using a light microscope (Olympus CX21, Japan) with a micrometer ruler (Eyarefe *et al*., 2008). The absorption area (AA, nm^2) was calculated using the formula:

$$AA = LV \times WV$$

Haematological and serum biochemical indices in experimental fish

Three fish were randomly selected per unit, and blood was collected via the caudal peduncle vein using tuberculin syringes with 24–gauge needles into heparinized tubes (Omitoyin *et al*., 2006). Samples were analyzed for haemoglobin concentration (Hb), packed cell volume (PCV), white blood cell count (WBC), erythrocyte count (RBC), and platelet counts. For PCV estimation, blood was placed in glass capillary tubes and centrifuged using a microhaematocrit centrifuge for 10 min. Methods described by Jain (1986) were used to determine erythrocyte indices, while Hb was measured by adding 2 µL of blood to 5 mL of Drabkin solution, allowing it to sit for 5 min before measuring colorimetrically (Vankampen and Zijlstra, 1961). White blood cells were determined using a Neubauer haemocytometer (Kaplow, 1955). Blood samples were centrifuged at 4 ºC to obtain plasma for biochemical analysis. Enzyme activities for alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured following Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was determined according to Tietz *et al*. (1983). Globulin (GLO), total proteins (TP), albumin (ALB), blood urea nitrogen (BUN), and creatinine were determined using the methods of Svobodová *et al*. (1991).

Determination of oxidative stress biomarkers

The livers of 2 fish per replicate were removed and held on ice. Half a gram of the liver was macerated in physiological saline and centrifuged for 10 min at 3,000 rpm (Ilavazhahan *et al*., 2012). The supernatants were stored at -20 ºC before analysis. These were analyzed for reduced glutathione (GSH), catalase activity (CAT), malondialdehyde (MDA), glutathione s-transferase (GST), superoxide dismutase (SOD) and glutathione peroxidase (GPx) following standard procedures (McCord and Frdovich, 1969; Habig *et al*., 1974; Jollow *et al*., 1974; Vashney and Kale, 1990 and Rajaraman *et al*., 1998, respectively). Total protein was determined using the Folin phenol method (Lowry *et al*., 1951).

Statistical analysis

Data were subjected to homogeneity tests of variance using Levene's test. Descriptive statistics and one-way analysis of variance (ANOVA) were used to determine the effects of EMM. Means were separated using the Duncan's Multiple Range Test at a significance level of 5% ($\alpha = 0.05$) with the aid of IBM SPSS version 20.

RESULTS

The crude extract yield from *Monodora myristica* seeds using ethanol as a solvent ranged from 6.2 to 6.8% in this study. The extracts were positive for alkaloids, flavonoids, saponin, and tannins, with a notably high presence of glycosides. After 12 weeks of rearing, fish fed EMM-supplemented diets showed improved growth as EMM concentration increased, although growth declined at the highest concentration (0.20%, M4). Growth parameters, including final weight, mean weight gain, and specific growth rate, followed similar trends, being higher in M2 and M3 groups compared to the control and M1 groups, although differences were not statistically significant ($p \geq 0.05$). Growth declined when EMM concentration reached 0.20% (M4). Feed intake was highest in the group fed with 0.15% EMM, while the FCR varied, tending to be lower in the and higher in M4 (1.48±0.05). A similar trend was observed for the protein efficiency ratio, with the M2 group showing the highest value and the M4 group the lowest. The survival rate was significantly improved in the M2 (0.10% EMM) and M3 (0.15% EMM) groups compared to the control. However, at the 0.20% EMM concentration, survival rates slightly decreased and were not significantly different from the control group (Table 2).

Table 2. Growth performance and nutrient utilization parameters of *Clarias gariepinus* fed *Monodora myristica* extract (EMM) at different concentrations.

Parameters	M0 (0%)	M1 (0.05%)	M2 (0.10%)	M3 (0.15%)	M4 (0.20%)
Initial weight (g)	2.28±0.04	2.23±0.01	2.33±0.06	2.32±0.04	2.45±0.05
Final weight (g)	18.26±0.56^{ab}	17.12±1.59^{ab}	19.42±0.98^{a}	19.57±0.63^{a}	16.00±0.35^{b}
Mean weight gain (g)	15.97±0.59^{ab}	14.89±1.61^{ab}	17.08±0.96^{a}	17.25±0.60^{a}	13.55±0.34^{b}
Specific growth rate (%g·day^{-1})	2.97±0.06^{a}	2.90±0.13^{ab}	3.02±0.17^{a}	3.05±0.04^{a}	2.68±0.03^{b}
Feed intake (g)	20.60±0.17^{b}	20.83±0.65^{b}	20.85±0.56^{b}	22.50±0.37^{a}	20.06±0.39^{b}
Feed conversion ratio	1.29±0.05^{ab}	1.42±0.10^{ab}	1.22±0.04^{b}	1.31±0.06^{ab}	1.48±0.05^{a}
Protein efficiency ratio (%)	2.21±0.09^{ab}	2.03±0.150.15^{ab}	2.33±0.09^{a}	2.19±0.11^{ab}	1.93±0.65^{b}
Survival rate (%)	86.67±1.67^{b}	88.33±1.67^{ab}	91.00±3.78^{a}	91.00±4.58^{a}	88.33±1.67^{ab}

Note: Mean±SD in each row, superscripted with different lowercase letters, denotes significant differences ($p<0.05$) among the means.

The villi height and width, as well as crypt depth and width, of *Clarias gariepinus* fed EMM-supplemented diets were similar ($p \geq 0.05$) across all EMM concentrations (Figure 1). The width of the villus ranged from 207.22 µm in the M0 group to 282.31 µm in the M2 group. The absorption areas in fish fed 0.10%, 0.15% and 0.20% EMM were higher compared to the control and M1 groups.

Changes in blood parameters were observed in fish fed EMM-supplemented diets. PCV, Hb RBC, Lymphocytes, heterophils, and MCV followed similar trends, with significant increases ($p<0.05$) in PCV, Hb, RBC, and lymphocytes, or declines in heterophils and MCV in the M1, M2, and M4 groups, while the M3 group showed values similar to the control ($p \geq 0.05$). Blood platelet counts increased significantly ($p<0.05$) in all EMM-treated groups compared with the control, except for the M3 group (Table 3). A significantly lower MCHC value was recorded in fish fed 0.2% EMM. Leukocyte, monocyte, and basophil counts did not differ significantly across treatments.

Changes in blood parameters were observed in fish fed diets supplemented with EMM, with a few exceptions. Specifically, blood protein, albumin, globulin, ALP, BUN, and creatinine levels increased, except for the M3 group (Table 4). AST and ALT levels were significantly higher in the control group, except for M1. However, the AG ratio did not differ significantly across all treatment and control groups.

The assay of antioxidant enzymes and lipid peroxidation in the liver of fish fed experimental diets showed significant increases in total protein, GPx, GSH, GST, and CAT ($p<0.05$) in EMM-supplemented groups, with some exceptions. The levels of malondialdehyde, on the other hand, were significantly lower in fish fed EMM. Superoxide dismutase levels exhibited no significant differences across treatments.

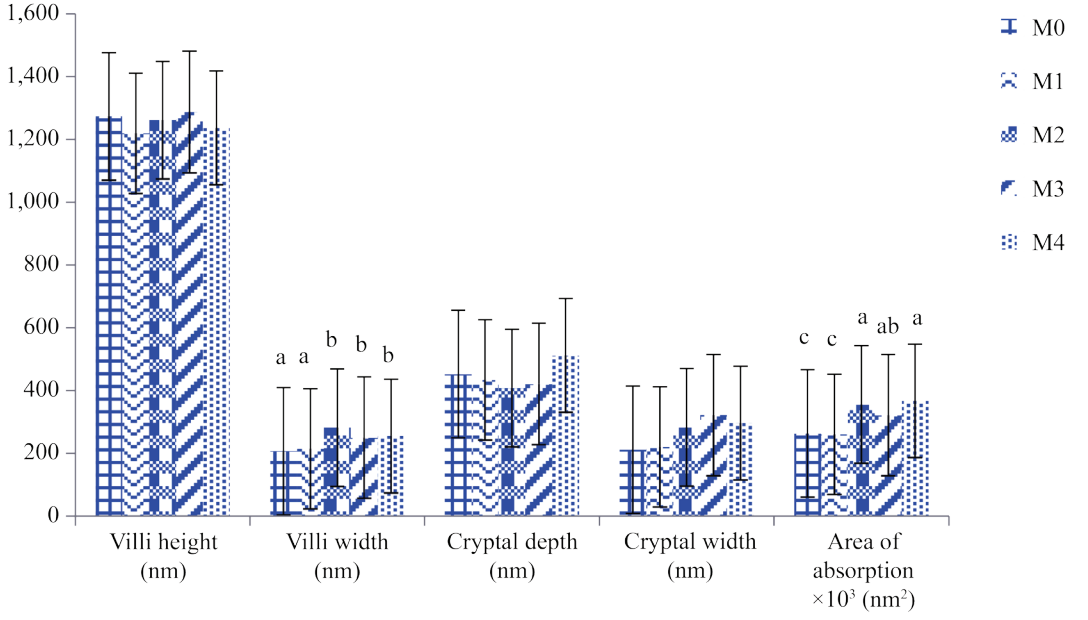

Figure 1. Gut morphometry of *Clarias gariepinus* fed diets supplemented with different concentrations of *Monodora myristica* extract. Different lowercase letters above the bars represent significant differences ($p<0.05$) among the means of each parameter. Parameters without superscripted letters denote non-significant differences ($p \geq 0.05$).

Table 3. Hematological indices of *Clarias gariepinus* fed diets supplemented with different concentrations of *Monodora myristica* extract.

Parameters	M0 (0%)	M1 (0.05%)	M2 (0.10%)	M3 (0.15%)	M4 (0.20%)
PCV (%)	26.00±1.00^{b}	34.50±0.50^{a}	34.00±2.00^{a}	28.00±2.00^{b}	37.50±0.50^{a}
Hb ($g \cdot dL^{-1}$)	8.65±0.05^{b}	12.15±0.55^{a}	12.85±0.15^{a}	9.00±0.60^{b}	9.15±1.35^{a}
RBC ($\times 10^{12} \cdot \mu L^{-1}$)	2.59±0.38^{b}	3.80±0.04^{a}	4.21±0.02^{a}	2.58±0.37^{b}	4.17±0.03^{a}
WBC ($\times 10^{9} \cdot \mu L^{-1}$)	11.95±1.05^{a}	15.42±3.42^{a}	14.30±2.65^{a}	13.37±1.52^{a}	16.67±3.75^{a}
Platelets ($\times 10^{3} \cdot \mu L^{-1}$)	153.50±9.50^{c}	256.00±18.00^{a}	237.50±14.50^{a}	185.00±11.00^{bc}	220.00±4.00^{ab}
Lymphocyte (%)	62.00±2.00^{b}	74.00±1.00^{a}	75.00±3.00^{a}	61.50±1.50^{b}	72.50±1.50^{a}
Heterophil (%)	29.50±2.50^{ab}	17.50±1.50^{c}	17.00±4.00^{c}	32.00±2.00^{a}	21.50±2.50^{bc}
Monocyte (%)	3.00±0.09^{a}	3.00±0.00^{a}	3.00±0.10^{a}	3.50±0.05^{a}	3.50±0.15^{a}
Basophil (%)	0.50±0.00^{a}	0.50±0.05^{a}	0.50±0.05^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MCV (Fl)	101.86±11.26^{a}	90.81±2.27^{b}	43.34±2.58^{c}	109.50±8.15^{a}	89.92±0.55^{b}
MCHC (%)	33.31±1.09^{a}	35.20±1.08^{a}	37.95±2.67^{a}	32.15±0.15^{a}	24.35±3.27^{b}
MCH (pg)	34.05±4.86^{a}	31.99±1.78^{a}	15.31±1.05^{c}	35.22±2.79^{a}	21.92±3.08^{a}

Note: PCV = Packed Cell Volume; Hb = Hemoglobin; RBC = Red blood cells; WBC = White Blood Cells; MCV = Mean Corpuscular Volume (femtoliter); MCHC = Mean Corpuscular Hemoglobin Concentration; MCH = Mean Corpuscular Hemoglobin (Picogram); Mean±SD in each row, superscripted with different lowercase letters, denotes significant differences ($p<0.05$) among the means.

Table 4. Serum biochemistry of *Clarias gariepinus* fed diets supplemented with different concentrations of *Monodora myristica* extract.

Parameters	M0 (0%)	M1 (0.05%)	M2 (0.10%)	M3 (0.15%)	M4 (0.20%)
Protein ($g \cdot dL^{-1}$)	7.50±0.10^{c}	9.95±0.25^{b}	10.90±0.10^{a}	5.15±0.15^{d}	10.15±0.35^{b}
Albumin ($g \cdot dL^{-1}$)	3.10±0.00^{bc}	3.75±0.05^{a}	4.25±0.05^{a}	2.55±1.35^{c}	4.30±0.30^{a}
Globulin ($g \cdot dL^{-1}$)	4.40±0.10^{c}	6.20±0.30^{a}	6.25±0.15^{a}	2.60±1.20^{d}	5.85±0.50^{b}
AG ratio ($g \cdot dL^{-1}$)	0.71±0.02^{b}	0.61±0.04^{b}	0.64±0.02^{b}	0.96±1.24^{a}	0.74±0.05^{b}
AST (µL)	218.50±5.50^{a}	211.50±3.50^{a}	193.00±4.00^{b}	178.50±1.50^{b}	190.50±5.00^{b}
ALT (µL)	49.50±3.50^{a}	40.00±10.00^{a}	31.50±1.50^{b}	29.50.0.50^{c}	38.50±10.50^{ab}
ALP (µL)	206.50±6.50^{c}	225.50±63.50^{b}	219.00±65.00^{b}	195.00±11.00^{c}	246.50±21.50^{a}
BUN ($mg \cdot dL^{-1}$)	6.10±0.10^{b}	8.60±0.10^{a}	8.65±0.05^{a}	5.65±0.35^{b}	8.20±0.20^{a}
Creatinine ($mg \cdot dL^{-1}$)	0.60±0.00^{c}	1.05±0.05^{ab}	1.15±0.05^{a}	0.55±0.05^{c}	1.00±0.00^{b}

Note: ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; AG = albumin globulin ratio; BUN = blood urea nitrogen; Mean±SD in each row, superscripted with different lowercase letters, denotes significant differences ($p<0.05$) among the means.

Table 5. Liver oxidative biomarkers in *Clarias gariepinus* fed diets supplemented with different concentrations of *Monodora myristica* extract.

Parameters	M0 (0%)	M1 (0.05%)	M2 (0.10%)	M3 (0.15%)	M4 (0.20%)
TP (µg·dL^{-1})	14.20±0.22^{c}	16.61±0.17^{b}	18.66±0.23^{a}	17.07±0.04^{b}	14.09±0.46^{c}
GPx (units·mg protein^{-1})	8.73±0.33^{b}	11.41±1.10^{a}	13.01±0.16^{a}	12.06±0.40^{a}	11.23±0.81^{a}
GSH (µg·mL^{-1})	216.12±15.90^{c}	474.03±9.99^{b}	453.52±21.96^{b}	636.67±36.50^{a}	260.01±27.99^{c}
GST (units·mg protein^{-1})	35.72±8.28^{c}	48.35±0.03^{bc}	47.17±1.73^{bc}	68.17±0.17^{a}	53.34±5.24^{ab}
MDA (units·mg protein^{-1})	5.29±1.71^{a}	4.04±0.54^{b}	4.44±2.21^{b}	4.09±2.51^{b}	3.95±0.00^{b}
SOD (units·mg protein^{-1})	2.00±0.02^{ab}	2.57±0.72^{a}	2.51±0.04^{a}	2.77±0.18^{a}	1.20±0.46^{b}
CAT	10.77±1.72^{b}	14.11±0.66^{a}	12.66±0.23^{ab}	10.57±0.54^{b}	14.09±0.46^{a}

Note: TP = total protein; GPx = Glutathione peroxidase; GSH = Reduced glutathione; GST = Glutathione S-Transferase; MDA = Malondialdehyde; SOD = Superoxide dismutase; CAT = Catalase activity; Mean±SD in each row, superscripted with different lowercase letters, denotes significant differences ($p<0.05$) among the means.

DISCUSSION

Alkaloids, flavonoids, saponin, tannins, and glycosides were phytochemicals found in the ethanolic seed extracts of Monodora myristica in this study. These compounds are products of metabolic reactions during the life cycle of plants and are classified as secondary metabolites (Okwu, 2004). These phytochemicals impart antioxidative, pharmacological, and biochemical properties to the extract. In this study, although glycosides were present, they did not significantly affect growth, despite their reported role in growth regulation (Benito, 2022). According to this author, the absorption of glycosides in the digestive tract is influenced by their chemical structure and the type of bonds they contain. However, the structural properties of the phytochemicals were not explored in the current study.

While the mean weight gain and specific growth rate were slightly higher in fish fed 0.1% and 1.5% EMM diets, these differences were not statistically significant when compared to the control group. The marginal growth improvement observed in the M2 and M3 groups may be attributed to the duration of administration of bioactive compounds in the EMM to the fish. However, fish fed more than 1.5% of the extract showed reduced weight gain, suggesting that the components of the crude extract may have an antinutritional effect at higher concentrations. Peterson *et al*. (2014) found that phytochemicals did not boost growth performance in channel catfish (*Ictalarus punctatus*). In contrast, Lawhavinit *et al*. (2011) reported that ethanolic turmeric extracts improved weight gain when included in the diet of white shrimp at 15 g·kg^{-1}. With the exception of the group fed 1.5% extract, feed intake did not vary significantly across the groups in this study, indicating that the flavor and palatability of the diet were likely unaffected by the additive. This contrasts with the findings of Kroismay *et al*. (2006), who observed that dietary supplementation with phytogenic compounds improved diet palatability due to the presence of certain flavonoids. The growth indices observed in this study differ from those reported by Abdel-Tawwab *et al*. (2018) and Omitoyin *et al*. (2019), who found significantly improved growth and nutrient utilization when Nile tilapia (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) were fed clove basil leaf extract, and guava leaf extract. The inclusion of compounds such as tannins and flavonoids according to the scientists, was attributed to the observed growth improvement in their studies.

When compared to the control, fish fed EMM-supplemented diets exhibited higher surface area of absorption. The modulation of gut architecture observed in this study contrasts with the findings of Merrifield *et al*. (2010), who reported larger

villi dimensions in rainbow trout fed *Pediococcus acidilactici* extracts, which contributed to improved growth. However, the results of this study align with those of Omitoyin *et al*. (2019), who noted that phytobiotics help maintain or improve normal intestinal architecture, thereby enhancing the surface area available for intestinal absorption.

Blood and serum indices serve as indicators of the health status of cultured organisms. The increased packed cell volume (PCV) and platelet count in fish fed EMM-supplemented diets demonstrate the plant's beneficial effects on fish health. Bioactive plant compounds have been shown to boost blood cell counts in various fish species, enhancing immune system function and natural defense mechanisms (Ajani *et al*., 2020). Most of the hematological parameters in this study were within the acceptable ranges for warm-water fish species (Nwanna *et al.*, 2014).

Serum biochemical profiles provide insights into the immunity status of cultured species. These profiles also reflect the health and function of the liver and kidneys, as well as the fatty acid composition in the blood. In this study, fish fed EMM-supplemented diets showed reduced levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating healthy liver function. Similarly, Ajani *et al*. (2020) reported a decrease in ALT and AST in the liver of Nile tilapia fed turmeric (*Curcumin longa*) powder. Jahanjoo *et al*. (2018) also found no liver injury and a reduction in hepatic amino acid utilization. The increase in alkaline phosphatase levels observed in fish fed EMM-supplemented diets suggests that the chemical components of the extracts were well absorbed and that liver cell function remained normal (Omitoyin *et al*., 2019).

Fish fed diets supplemented with EMM showed a significant increase in albumin, globulin, creatinine, and blood urea nitrogen levels, except in the M3 group. The elevated total protein (TP), glutathione peroxidase (GPx), glutathione (GSH), and glutathione-S-transferase (GST) levels suggest activation of antioxidant protection, which is essential for healthy fish during a typical feeding cycle. The modulation of the immune system in fish through the use of plant extracts may be attributed to the phytochemicals' ability to enhance bactericidal activities, complement lysozyme activities, and stimulate natural killer cells in fish (Dadras *et al*., 2019). Fish with higher GPx levels are expected to have better innate immune systems, indicating stronger antimicrobial activity by white blood cells (Ajani *et al*., 2020). In this study, the total protein levels were within the acceptable range for freshwater species (Das *et al*., 2012).

Malondialdehyde (MDA), an end product of lipid peroxidation induced by oxygen free radicals, is a key indicator of oxidative stress. An increase in MDA is typically associated with cellular damage (Omitoyin *et al*., 2019). The results of this study showed a slight reduction in MDA values in fish fed with the extract, compared to the control group, suggesting a reduction in oxidative stress.

CONCLUSIONS

In conclusion, the ethanolic extract of *Monodora myristica*, particularly at a lower concentration (0.10%) can marginally improve growth performance and feed utilization, while significantly enhancing survival rates. However, higher concentrations showed negative effects, likely due to the presence of anti-nutritional factors. The improved growth observed in fish fed lower concentrations of the extract can be attributed to enhanced nutrient absorption, as indicated by the increased absorption surface area in the gut. Additionally, the inclusion of 0.10%-0.15% crude extract of *M. myristica* in the diet of *Clarias gariepinus* for 12 weeks improved immune status, as evidenced by the positive changes in oxidative biomarkers and other blood and serum indices.

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