Aquaculture Studies, 25(5), 245-253, AQUAST2394
http://doi.org/10.4194/AQUAST2394

RESEARCH PAPER

Effect of Almond *Terminalia Catappa* leaf Extracts on Growth, Gut Morphometry, Immunocompetence and Resistance of *Heterobranchus longifilis* to *Pseudomonas Aeruginosa*

Olugbenga Orisasona[1,*], Abimbola Olumide Adekanmbi[2], Bisi Patience Akinrinade[1], Oyebola Oluwafunke Taiwo[3]

[1]Obafemi Awolowo University, Faculty of Agriculture, Department of Animal Sciences, Ile-Ife Osun State, Nigeria (220282).
[2]University of Ibadan, Faculty of Science, Department of Microbiology, Ibadan Oyo State, Nigeria.
[3]University of Ibadan, Faculty of Renewable Natural Resources, Department of Aquaculture and Fisheries Management, Ibadan Oyo State, Nigeria.

How to Cite

Orisasona, O., Adekanmbi, A.O., Akinrinade, B.P., Taiwo, O.O. (2025). Effect of Almond *Terminalia Catappa* leaf Extracts on Growth, Gut Morphometry, Immunocompetence and Resistance of *Heterobranchus longifilis* to *Pseudomonas aeruginosa*. *Aquaculture Studies*, *25(5), AQUAST2394*. http://doi.org/10.4194/AQUAST2394

Article History
Received 21 January 2025
Accepted 30 April 2025
First Online 07 May 2025

Corresponding Author
E-mail: osasonagbenga@gmail.com

Keywords
Indian almond
African catfish
Growth
Solvent
Immune system

Abstract

This work evaluated the outcome of dietary ethanol (EE) and aqueous (AE) extracts of *Terminalia catappa* (Tropical almond) on growth, survival, oxidative stress, and resistance of *Heterobranchus longifilis* (Bagrid catfish) fingerlings to *Pseudomonas aeruginosa*. Five diets with different concentrations of *T. catappa* extracts (AL1, 0.0% extract; AL2, 0.5% EE; AL3, 1.0% EE; AL4, 0.5% AE and AL5, 1.0% AE) were formulated. Triplicate groups of *H. longifilis* (3.82±0.05 g) were fed diets at 3% body weight twice daily for 70 days. Results indicated that EE were richer in bioactive compounds including total phenolics, tannins, alkaloids, and flavonoids compared to AE. However, ethanol extracts negatively impacted feed intake and growth performance, particularly at higher concentration. Fish fed with aqueous extracts exhibited improved growth, higher hematocrit values, and enhanced antioxidant enzyme activity. Survival rates were highest in fish fed with aqueous extracts, particularly at 1.0% concentration. Fish fed with aqueous extracts showed increased resistance to *Pseudomonas aeruginosa* challenge, as evidenced by the higher relative percentage survival (RPS). These findings suggest that *Terminalia catappa* aqueous extracts can positively influence growth, health, and disease resistance of *Heterobranchus longifilis*. The study highlights the importance of considering extraction solvent when using plant-based extracts in aquaculture feed.

Introduction

The intensification of aquaculture systems often face challenges related to disease management and compromised immune systems, mostly controlled using antibiotics and other synthetic compounds (Magnadottir, 2010; Irshath *et al*., 2023). These often lead to reduced productivity, and the accumulation of antibiotic residues in the products and environment. Accumulation of antibiotics result from the abuse of drugs and chemicals in culture facilities accompanied with increased risk of development of antimicrobial resistance in the aquatic environment (Chowdhury et al*.,* 2022). To overcome these challenges, there is a growing focus on developing natural compounds as alternatives to synthetic chemicals.

Herbal plants and extracts which are rich in bioactive compounds, offer promising solutions for sustainable aquaculture. These bioactive compounds, including tannins, flavonoids, alkaloids, terpenoids, and steroids, possess antimicrobial, immune-stimulatory, and growth-promoting properties (Chakraborty et al*.,* 2013: Reverter et al*.,* 2014). Unlike synthetic chemicals, plant extracts are generally considered safer for the environment and human health. Previous work has shown the potentials in herbal plants and the extracts in promoting growth and improving survival in fish species (Ajani et al., 2020; Omitoyin et al., 2019; Abdel-Tawwab *et al.*, 2018; Abdel-Tawwab and Abass, 2017;). The ease of access and relative safety for environment and humans when compared with synthetic compounds, makes plant extracts acceptable (Reverter et al*.,* 2014;

Chakraborty et al*.,* 2013). Extracting the bio-compounds in the plant will allow for easy manipulation of extracts and improve the efficiency when fed or administered to fish. However, the methods of extraction, solvent used and the plant parts are significant to the quality of extract. Bae et al*.* (2012) reported higher emodin levels in ethanol extracts of *Rumex acetosa* than in the water extract, causing the former to have greater anti-ulcer and inhibitory activities. According to Sarwar et al*.* (2012), there was variation in the efficacy of extracts from almond shell as a result of different extracting solvents.

Terminalia catappa, a tropical almond tree, has been traditionally used for its medicinal properties. Its extracts contain bioactive compounds including flavonoids, phenolic acids, and tannins namely, punicalagin, punicalin, geranin, granatin, corilagin (Ahmed et al*.,* 2005), with potential benefits for aquaculture. However, despite the potential benefits of *T. catappa* extracts, the specific effects of *T. catappa* extracts on *H. longifilis* health and growth remain understudied.

Heterobranchus longifilis is one of the most farmed species from the genera Clarias and Heterobranchus (Robert et al*.*, 2019). The culture of this species is very important for sustainable aquaculture production considering the acceptability, adaptation to intensive culture and fast growth. The all-out culture of this species is often plagued by disease outbreaks mostly in the seed production stage. An organism culpable in disease incidence in *H. longifilis* production is the *Pseudomonas aeruginosa* (Akinwande et al., 2016). It has strong tendency to become opportunistic under conditions of stress. Attack of *P. aeruginosa* results in the systemic infection known as *Pseudomonas* septicemia, usually manifested by hemorrhagic ulceration of the skin, peeling of the skin, abdominal distension, exophthalmia and high mortality (Raj *et al*., 2019; El-Bahar et al., 2019). For sustainable fish production aimed at enhancing food and nutrition security, fish must be protected from pathogenic organisms in an ecofriendly manner using bioactive compounds properly extracted. This study aims to weigh up the impact of dietary *Terminalia catappa* extracts on the growth indicators, gut morphology, hematology, and disease resistance of *Heterobranchus longifilis* fingerlings. By comparing ethanol and aqueous extracts, the study will investigate the outcome of these solvents on the bioactive compounds and biological activity of the extracts.

Materials and Methods

Terminalia catappa Extraction and Phytochemical Screening

Indian almond leaves collected from trees around Obafemi Awolowo University were identified at the herbarium in the Department of Botany. Leaf particles obtained from air-dried (at 30˚C for 96 hours) and milled samples were soaked at 0.25 kg/L of distilled water or ethanol (80% aqueous ethanol) as solvents, for 48 hours and shaking occasionally (Omitoyin et al., 2019), using the cold maceration method. Extracts are separated by filtration using Wattman paper and then concentrated with rotary evaporator at 150 revolution per minute (rpm) at temperature between 37-40˚C. Each extract was air dried to remove any residual solvent (Ingle et al., 2017) and yield ranged from 7.2 to 9.2%. The extracts were subjected to quantitative and qualitative Phyto-screening according to Yadav et al*.,* (2014).

Experimental Diets, Procedure and Design

Five experimental diets containing 40% crude protein, with diet al1 containing no extract (control), AL2 (0.5% ethanol extract), AL3 (1.0% ethanol extract), AL4 (0.5% aqueous extract) and AL5 (1.0% aqueous extract) were produced. The feedstuffs in Table 1, were weighed individually, and mixed thoroughly to

Table 1. Gross composition of *Terminalia catappa* diets in g/kg fed *Heterobranchus longifilis* for 70 days

Feed ingredients	AL1	AL2	AL3	AL4	AL5
Fish meal	188.5	188.5	188.5	188.5	188.5
Soybean meal	377	377	377	377	377
Groundnut cake	188.5	188.5	188.5	188.5	188.5
Maize	95.5	93	90.5	93	90.5
Wheat offal	95.5	93	90.5	93	90.5
Lysine	5	5	5	5	5
Methionine	5	5	5	5	5
Di-calcium phosphate	5	5	5	5	5
Starch	5	5	5	5	5
Salt	5	5	5	5	5
Vegetable oil	20	20	20	20	20
Vitamin premix	10	10	10	10	10
Extract	0	5	10	5	10
Proximate composition					
Crude protein	39.79	40.10	40.09	40.14	40.10
Crude fibre	4.01	3.47	3.97	4.20	4.15
Ether extract	5.81	5.24	5.81	5.07	5.37
Ash	5.11	5.31	5.43	5.17	5.11
Moisture	8.89	8.90	9.00	8.91	9.11

homogenous masses. With a 2 mm die, the masses were pelletized in a Hobart A200 machine. The pellets were dried in unheated air and packed into well labeled air-tight plastic bags. Feeds were produced every three weeks.

Heterobranchus longifilis fingerlings were procured from a reputable fish farm and acclimatized for 14 days and fed the control diet during this period. Thereafter, 20 *H. longifilis* each (3.82±0.05 g per fish) were randomly allotted into 15 rectangular plastic aquaria (0.42 m x 0.29 m x 0.25 m) with water volume kept at 0.18m level (20L). Feeding to apparent satiation was done twice daily at 7 hours interval for 70 days. For each feeding time, the administered quantity was recorded and leftovers removed daily, with the difference in the two values accounting for daily feed intake (Helland et al*.,* 1996). Fish were weighed fortnightly. The Combine probe YSI (Model 57) was used to monitor dissolved oxygen and temperature. The Photoic 20 pH meter was used to measure pH, while values of ammonia and nitrite was determined using the commercial test kit (HACH Fish Farm Testing Kit, Model FF-1A, USA).

Determination of Body Growth and Utilization of Dietary Nutrients in *Heterobranchus longifilis* Fed *Terminalia catappa* Diets

The weights of fish were measured bi-weekly using a model C5000 scale. After 70-day feeding, indicators of growth and nutrient utilization, and survival rate (%) were calculated. The following growth parameters were calculated (Castel and Tiews, 1980).

Feed Consumed (g)

Sum of feed intake for 70 days.

Weight Gained (g)

Is equal to final weight of fish (W2) minus initial weight (W1)

$$Weight\ gain\ (g) = W2 - W1$$

Average Daily Weight Gain (g)

Is equal to the weight gain divided by 70 (Feeding days)

$$Average\ daily\ weight\ gain\ (g) = \frac{weight\ gain\ (g)}{days\ of\ feeding}$$

Specific Growth Rate (SGR)

$$Specific\ growth\ rate\ (SGR) = \frac{logw_2 - logw_1}{T_2 - T_1} \times 100$$

$\log_e$ = Natural logarithm

T2-T1= 70

Food Conversion Ratio (FCR)

$$FCR = \frac{feed\ intake(g)}{weight\ gain(g)}$$

Survival Rate (%)

$$Survival\ rate = \frac{No\ of\ fish\ at\ T_2}{No\ of\ fish\ at\ T_1} \times 100$$

Evaluation of Gut Morphometric of *Heterobranchus longifilis* Fed *Terminalia catappa* Diets

From each experimental treatment, 6 fish were taken randomly and tranquilized with 30mg/l buffered trichinae methane sulfonate. The intestines were excised immediately for histological examination, with gut preparations on slide done according to Drury et al. (1967) and Cullin (1974). The length and width of villi (VL and VW respectively) and depth of the crypt were measured in cm and absorption area (AA) calculated in cm^2 with the aid of a light microscope (Hex40) (CX21 Olympus, Japan) having a micro meter rule (Eyarafe et al*.,* 2008).

Hematological and Serum Biochemical Analysis of *Heterobranchus longifilis* Fed *Terminalia catappa* Diets

After 70 days of feeding trials, six fish selected randomly from each treatment were serially bled (Omitoyin *et al.,* 2019). A set of bottles with sodium heparinate (20 U/L) received samples for blood analysis while a second set received for serum biochemical analysis. For the estimation of packed cell volume (PCV), fresh blood was centrifuged in a microhaematocrit centrifuge at 10,000 rpm for duration of 5 min and measured. A micropipette was used to collect 2 microlitres of the sample and added to 5 ml of Drabkin solution and allowed to stand for 5 min and colorimetric readings taken for hemoglobin estimation (Vankampen and Zijlstra, 1961). Methods described by Jain (1986) were used to determine erythrocytic indices, while the Neubauer haemocytometer method described by Kaplow (1955) was used to measure white blood cells (WBC). The Reitman and Frankel (1957) colorimetric method was used to determine ALT (alanine aminotransferase) value, while method described by Tietz *et al.* (1983) was used for ALP (alkaline phosphate) estimation. Albumin (ALB), globulin (GLO), creatinine (CREAT) and blood urea nitrogen (BUN) were determined (Svobodova et al*.,* 1991).

Determination of Oxidative Stress İndices in *Heterobranchus longifilis* Fed T*erminalia catappa* Diets

From each treatment, livers from 6 fish randomly picked were excised into ice before analyzing for oxidative stress. 0.5 g of liver samples per treatment macerated in physiological saline solution were

centrifuged (3,000 rpm; 10 min) (Ilavazhahan et al., 2012). Before analyzing, the supernatants were placed bottles and frozen at -20°C. Superoxide dismutase (SOD), total protein, glutathione peroxide (GPx) and glutathione s-transferase (GST) were determined according to Misra and Fridovich (1972), Lowry et al. (1951), Aebi (1984), and Habig et al. (1974) respectively.

Preparation of Bacterial Strain of *Pseudomonas aeruginosa* and Challenge Test

Pseudomonas aeruginosa isolate was sub-cultured for 24 hours (37°C) in tryptic soya broth. After centrifugation, bacterial pellets were then taken and then suspended in sterile PBS solution (physiological buffer saline) and adjusted to ($1x10^7$cfu/ml) in phosphate buffered saline (PBS). Twenty fish representing each treatment were randomly taken and divided into two groups (A and B). They were then fasted for 24 hours before injection. 0.2 ml PBS containing virulent *P. aeruginosa* was intraperitoneally injected into group A (Misra et al., 2006). While 0.2 ml of saline solution was intraperitoneally injected into group B (control). Fish were observed for 2 weeks during which mortalities and strange clinical signs were recorded. Using the Kocour et al. (2007) equation, relative percentage survivals (RPS) in challenged fish were determined:

$$RPS\% = \frac{No\ of\ surving\ fish\ after\ challenge}{No\ of\ fish\ injected\ with\ bacteria} \times 100$$

Statistical Analysis

Data obtained were tested for homogeneity of variance using the Levene's test. One-way analysis of variance (ANOVA) was used to analyze data and determine the impact of *Terminalia catappa* extract on *H. longifilis*. Means were separated using the Duncan Multiple Range Test at 5% probability level. Analysis was carried out using SPSS version 20.

Results

Results of the qualitative analysis of ethanolic and aqueous extracts of Indian almond leaf is presented (Table 2), with chalcones, quinine, anthocyanins, and emodin all absent in ethanolic extract, but present in aqueous extract. Di-terpenes was detected in ethanolic extract but absent in aqueous extract while cardiac glycosides was absent in both extracts. Except for saponin, phytochemicals were more concentrated in ethanolic extract of *Terminalia catappa* leaf (Table 3). Flavonoids in aqueous extract appear very negligible when compared with the value recorded for ethanolic extract.

Table 2. Qualitative phytochemical screening of Ethanolic and Aqueous extract of *Terminalia catappa* leaf

Phytochemicals	Inference	
	Ethanolic	**Aqueous**
Saponin (Froth's Test)	**+ve**	**+ve**
Tannin (Braymer's Test)	**+ve**	**+ve**
Flavonoid (Lead acetate Test)	**+ve**	**+ve**
Steroid (Salkowaski's Test)	**+ve**	**+ve**
Terpenoid (Salkowaski's test)	**+ve**	**+ve**
Coumarin (Reaction with 10 % NaOH)	**+ve**	**+ve**
Chalcones (Ammonium Hydroxide)	**- ve**	**+ve**
Quinone (Hcl)	**-ve**	**+ve**
Anthocyanins (Reaction with Acid and Ammonia)	**-ve**	**+ve**
Alkaloid (Hager's Test)	**+ve**	**+ve**
Cardiac Glycosides (Legal's Test)	**-ve**	**-ve**
Phenols (Ferric Chloride's Test)	**+ve**	**+ve**
Di-terpenes (Cupric Acetate's Test)	**+ve**	**-ve**
Protein (Conc HNO_3)	**+ve**	**+ve**
Emodin (With Benzene)	**-ve**	**+ve**

Table 3. Quantitative Phytochemical Screening of Ethanoic and Aqueous Extract of *Terminalia catappa* leaf

Phytochemical	Concentration	
	Ethanoic extract	Aqueous extract
Total Phenolics (mg/g GAE)	89.139±0.006	42.46±0.000
Tannin (mg/g GAE)	5.862±0.008	2.10±0.004
Alkaloid (mg/g)	13.66±0.030	10.30±0.030
Flavonoid (mg/g RE)	97.259±0.257	2.741±0.026
Saponin (%)	1.34±0.010	2.04±0.040

Growth indices in *H. longifilis* fed aqueous extract diets and the control group did not vary significantly as shown in Table 4. Mean final weight gain ranged from 8 g in fish fed 0.5% ethanolic extract of *T. catappa* leaf through 23.17 g in those fed 1% aqueous extract to 25.67 in the control. Feed intake was statistically similar in the AL1, AL4 and AL5 groups with significantly higher (P<0.05) values than *H. longifilis* fed ethanolic extracts. Feed conversion ratio and specific growth rate followed the same trend in treatments. The rate of survival was significantly higher (P<0.05) fish fed aqueous extracts of *T. catappa*. Survival range d from 51.67% in *H longifilis* fed 1.0% alcohol extract to 95% in fish fed 1% aqueous extract of Indian almond leaf.

Table 5 reveals no significant variations in villi height across treatments, while villi width in *H. longifilis* fed the control and 0.5% aqueous extract was significantly higher. Higher cryptal depths were observed in control. However, cryptal width was significantly higher in the aqueous groups and the control. The gut absorption area was however not significantly affected.

Packed cell volume in all experimental fish ranged from 22.00 g/dl in the group fed 1% ethanolic extract of *Terminalia catappa* leaf to 31.00 g/dl in groups fed 0.5% aqueous extract of leaf (Table 6). Significantly lower (P>0.05) PCV's were observed in the ethanolic extract groups. Hemoglobin in experimental fish is lower in fish fed 1% ethanolic extract compared to others. Red blood cell counts was higher (P<0.05) in AL1, AL2 and AL4. However, there was no significant difference in the white blood cells across treatments. For granulocytes, basophils were statistically similar across treatment, while eosinophil was significantly reduced in AL3, AL4 and AL5. Mean corpuscular volume and mean corpuscular hemoglobin in *H. longifilis* fed 1% aqueous or ethanolic leaf extracts were significantly higher.

Total protein was significantly higher in fish fed *T catappa* extracts compared to the control group. Fish fed aqueous leaf extracts displayed the highest values

Table 4. Growth, Nutrient utilization and survival of *Heterobranchus longifilis* fed *Terminalia catappa* based diet for 70 days.

Parameter	AL1	AL2	AL3	AL4	AL5
Initial weight (g)	3.70±0.12	3.87±0.07	3.90±0.06	3.80±0.05	3.83±0.03
Final weight (g)	$29.33±1.33^{a}$	$8.00±0.58^{b}$	$11.33±0.88^{b}$	$24.00±3.46^{a}$	$25.67±1.76^{a}$
Feed intake (g)	$36.97±0.64^{a}$	$17.20±0.69^{b}$	$21.41±3.76^{b}$	$32.63±2.41^{a}$	$34.63±1.45^{a}$
Weight gain (g)	$25.67±1.42^{a}$	$4.13±0.52^{b}$	$7.43±0.85^{b}$	$20.20±3.46^{a}$	$23.17±1.51^{a}$
ADWG (g)	$0.37±0.02^{a}$	$0.06±0.01^{b}$	$0.11±0.01^{b}$	$0.29±0.04^{a}$	$0.33±0.02^{a}$
SGR (%g/day)	$1.31±0.04^{a}$	$0.45±0.04^{c}$	$0.67±0.04^{b}$	$1.15±0.08^{a}$	$1.23±0.03^{a}$
FCR	$1.45±0.05^{c}$	$4.27±0.43^{a}$	$2.89±0.48^{b}$	$1.68±0.22^{c}$	$1.50±0.04^{c}$
Survival rate (%)	$85.00±7.64^{b}$	$65.00±2.89^{bc}$	$51.67±21.28^{c}$	$91.33±4.41^{a}$	$95.00±2.89^{a}$

Table 5. Gut Morphometry of *Heterobranchus longifilis* fed experimental diet for 70 days

Parameters	AL1	AL2	AL3	AL4	AL5
Villi height(nm)	1434.70	1474.49	1470.54	1391.42	1359.70
Villi width(nm)	183.32^{a}	149.49^{b}	163.07^{b}	180.61^{a}	164.04^{b}
Cryptal depth (nm)	458.86^{a}	315.69^{b}	318.67^{b}	295.70^{b}	263.94^{c}
Cryptal width (nm)	187.46^{a}	148.67^{c}	163.34^{bc}	186.70^{a}	169.07^{b}
Muscle thickness(nm)	409.38^{a}	293.05^{bc}	359.16^{ab}	375.64^{a}	259.78^{c}
Area of absorption (nm^2)	266439^{a}	219935^{b}	239467^{ab}	250855^{ab}	224583^{ab}

Table 6. Hematological indices of *Heterobranchus longifilis* fed experimental diet for 70 days

Parameters	AL1	AL2	AL3	AL4	AL5
PVC (g/dl)	$30.00 ±1.55^{b}$	$27.20±0.58^{a}$	$22.00±0.58^{a}$	$31.00±0.58^{b}$	$29.00±0.58^{b}$
Hb (g/dl)	$9.83±0.27^{b}$	$9.57±0.22^{b}$	$7.80±0.44^{a}$	$10.03±0.26^{b}$	$9.57±0.22^{b}$
RBC (x 10^6/µl)	$2.86±0.08^{b}$	$2.91±0.04^{b}$	$1.78±0.08^{a}$	$3.00±0.09^{b}$	$1.76±0.06^{a}$
WBC (x 10^6/µl)	13.90 ±1.87	13.56±1.47	13.35±0.52	14.60±1.93	14.30±0.89
Platelet (x 10^3/µl)	$153.66 ±7.44^{a}$	$151.00±11.59^{a}$	$200.13±11.19^{b}$	$156.67±2.03^{a}$	$163.00±8.08^{ab}$
Lymphocytes (%)	$59.67±2.33^{bc}$	$59.67±0.33^{bc}$	$54.33±1.76^{a}$	$63.33±0.88^{c}$	$56.00±0.58^{ab}$
Heterocytes (%)	$32.67±2.91^{a}$	$32.67±0.67^{a}$	$38.67±1.76^{b}$	$30.00±1.15^{a}$	$39.33±0.89^{b}$
Monocytes (%)	$2.67±0.33^{a}$	$3.33±0.88^{a}$	$4.33±0.67^{b}$	$3.67±0.67^{a}$	$3.33±0.33^{a}$
Eosinophil (%)	$4.00±1.00^{b}$	$4.00±0.58^{b}$	$2.33±0.88^{ab}$	$2.67±0.67^{ab}$	$2.00±0.58^{a}$
Basophil (%)	$0.33±0.33^{a}$	$0.33±0.33^{a}$	$0.33±0.33^{a}$	$0.67±0.33^{a}$	$0.33±0.33^{a}$
MCV (Fl)	$104.97±1.40^{a}$	$99.57±2.20^{a}$	$124.30±2.69^{b}$	$103.42±1.95^{a}$	$136.99±6.23^{c}$
MCHC (%)	$32.81±0.46^{a}$	$32.98±0.29^{a}$	$32.43±0.61^{a}$	$32.36±0.23^{a}$	$32.45±1.04^{a}$
MCH (Pg)	$34.98±0.61^{a}$	$32.8±0.61^{a}$	$40.30±0.94^{b}$	$33.46±0.59^{a}$	$44.56±3.32^{b}$

for total protein as shown Table 7. Glutathione peroxidase were marginally higher in fish fed extracts, while glutathione S-transferase were significantly higher in fish fed either 1% aqueous or ethanolic extracts. Malondaldehyde was highest in fish fed 1% ethanolic extract and vary significantly from other treatments. Catalase was significantly higher in the control and fish fed ethanolic leaf extracts.

The pattern of death in fish challenged with *Pseudomonas aeruginosa* after feeding with *C catappa* leaf extracts for 70 days show the highest mortality and consequently the lowest relative percentage survival (RPS) was recorded in the control group. Conversely, the least mortalities and highest RPS were observed in the groups fed 1% aqueous leaf extract (Table 8).

Discussion

Water or ethanol extracts of plants are natural sources of flavonoids, polyphenols, saponin, alkaloids, coumarins, glycosides, tannins, vitamins and other bioactive compounds with antioxidative properties (Plaskova and Mlcek, 2023). These secondary metabolites function in the plants' defense mechanism against insects, microbes, and herbivores (Mithofer and Maffei, 2017). In this study, ethanolic extract of *Terminalia catappa* leaf did not contain chalcones, quinone, anthocyanins and emodin, which were all present in the aqueous extract. The reverse is the case for di-terpenes. Higher concentrations of biomolecules were observed in the ethanolic extract except for saponin which is higher in aqueous extract. The variations observed in the occurrence and concentration of biomolecules in aqueous and ethanolic extracts of *T. catappa* leaf may be as a result of the differences in the polarity of various biomolecules. According to Lim et al. (2019), polar molecules are efficiently extracted using water as a solvent, while less polar ones are better extracted using organic solvents or binary solvents. Most of the bioactive compounds like phenols are poorly soluble in water, but have higher solubility in organic solvents such as ethanol (Giacobbo et al., 2015). Binary solvent mixtures like aqueous ethanol are adjudged better for biomolecules with higher molecular weights' extraction (Do, 2014). In *Cadaba rotundifolia* Forssk leaf extracts, it was revealed that 80% ethanol extract scored the highest yield compared to extracts with water as solvent or pure ethanol (Gonfa et al., 2020).

The current study showed no significant differences in growth and nutrient utilization between the control group and the group fed diets with aqueous extracts of *T. catappa*. However, both groups had significantly higher values compared to the group fed diets with ethanolic extracts. This may be due to increased feed intake in fish on the control and aqueous extract-based diets, which are likely more palatable and acceptable than the ethanolic extract diets. The presence of residual solvents in the ethanolic extracts could have denatured certain biochemicals, as indicated by the absence of chalcones, emodin, quinones, and anthocyanins in these extracts. This aligns with findings from Suleiman et al. (2023), who reported improved growth performance in *Clarias gariepinus* fed aqueous rather than ethanolic moringa leaf extracts.

In this present study, higher concentrations of phenolics, tannins, alkaloids, and flavonoids—up to double the amounts in aqueous extracts—were observed in the ethanolic extracts. These elevated levels may have hindered nutrient utilization and thus growth, suggesting that excessively high concentrations of these biomolecules can act as antinutrients. An optimal balance of biomolecules appears essential for nutrient utilization and growth, as evidenced by the superior

Table 7. Mean values for oxidative biomarkers and liver enzymes in *Heterobranchus longifilis* fed *Terminalia catappa* extract based diets for 70 days.

Parameters	AL1	AL2	AL3	AL4	AL5
Total protein (µg/dl)	12.33 ± 0.08^{a}	15.43 ± 0.16^{b}	15.31 ± 0.10^{b}	16.19 ± 0.09^{c}	17.24 ± 0.38^{d}
GPx (units/mg protein)	10.81 ± 0.35^{a}	11.51 ± 0.57^{ab}	11.79 ± 0.37^{ab}	11.03 ± 0.16^{ab}	12.26 ± 0.54^{b}
GST (unit/mg protein)	39.24 ± 0.55^{a}	46.80 ± 6.05^{abc}	56.29 ± 0.06^{c}	42.74 ± 1.39^{ab}	51.41 ± 3.04^{bc}
MDA (µmol/mg protein)	1.95 ± 0.30^{a}	2.39 ± 0.28^{a}	5.62 ± 1.38^{b}	2.03 ± 0.31^{a}	1.35 ± 0.12^{a}
SOD (units/mg protein)	5.56 ± 0.18^{bc}	6.60 ± 0.59^{c}	3.60 ± 0.67^{ab}	4.01 ± 1.60^{b}	1.73 ± 0.26^{a}
Catalase	2.23 ± 0.31^{ab}	2.96 ± 0.35^{b}	2.01 ± 0.31^{a}	1.95 ± 0.34^{a}	1.72 ± 0.04^{a}

Table 8. Record of mortalities in *Heterobranchus longifilis* fed experimental *Terminalia catappa* leaf extract diet for 70 days and challenged with *Pseudomonas aeruginosa*.

Treatment	No of fishes infected	DAY													Total	%Mortality	RPS
		1	2	3	4	5	6	7	8	9	10	11	12	14			
AL1	10	0	0	0	2	2	2	2	0	0	0	0	0	0	8	80	20
AL2	10	1	0	3	0	0	0	0	0	0	0	0	0	0	4	40	60
AL3	10	4	0	0	0	0	0	0	0	0	0	0	0	0	5	50	50
AL4	10	0	0	0	1	0	0	0	0	0	0	0	0	0	2	20	80
AL5	10	0	0	0	0	0	0	0	0	0	0	0	0	0	1	10	90

outcomes in fish fed aqueous extract-based diets. Additionally, fish survival rates were significantly higher in the aqueous extract group, highlighting the potential health compromise in fish fed ethanolic extract-based diets. This study underscores the need for controlled biomolecule concentrations in dietary extracts for optimal fish health and growth.

In this present study, villi and cryptal widths were significantly higher in the control and fish fed 1% aqueous extract, while higher cryptal depths were observed in control and groups fed ethanolic extract. There was however no corresponding increase in the villi height and therefore, the gut area of absorption showed no significant variation.

The importance of blood indices as pointers to the health status of cultured fish, nutritional balance, and the conduciveness of the culture environment cannot be overemphasized. The present study showed no variations in the white blood cell across treatments, however packed cell volume and hemoglobin concentration were significantly reduced in fish fed ethanolic extract of *T catappa* leaf. A reduction in the values of the very important indicators attests to the detrimental effect of higher concentrations of biomolecules in the ethanolic extracts. While previous studies revealed plants bioactive compounds increasing packed cell volume and hemoglobin in fish (Ajeel and Al-Faragi, 2013), this contradicts the result obtained for fish fed ethanolic extract, but is in agreement with the results in groups fed aqueous extract in this present study.

Terminalia catappa extracts promote the release of glutathione peroxidase, an antioxidant enzyme that neutralizes free radicals, thereby reducing lipid peroxidation. Fish fed diets containing *T. catappa* extract showed slightly higher levels of this enzyme. Additionally, a 1% inclusion of aqueous almond leaf extract provided better cellular protection, as indicated by higher glutathione S-transferase values in these groups. Galvez et al. (1996) attributed the antioxidative properties of *T. catappa* extracts to their phenolic compounds, which enhance the nonspecific immune response in fish. This finding aligns with Omitoyin et al. (2019), who reported that an aqueous extract of guava leaf (*Psidium guajava*) similarly enhanced the nonspecific immune system in *Oreochromis niloticus*.

Improved immune system is also affirmed from the results obtained from the challenge with *Pseudomonas aeruginosa*, where fish fed 0.5 and 1.0% aqueous extracts of almond leaf recorded 80-90% relative percentage survival compared to 20% in the control. The result is in agreement with findings of Ajani *et al*. (2020) and Abdel-Tawwab et al. (2018) when turmeric and clove respectively were fed to fish.

Conclusion

This study showed that although the concentration of bioactive compounds in the extracts of *Terminalia catappa* leaves are more using ethanol as solvent, growth and nutrient utilization, nonspecific immune system and survival of *Heterobranchus longifilis* were better enhanced when water is used as the extracting solvent.

Ethical Statement

This work was carried out in to compliance with internationally acceptable standards on humane care and use of animals for scientific purpose, under the strict monitoring of the Animal Research and Ethical Committee (AREC) with Protocol number: ARE-004/24. The clearance certificate is uploaded.

Funding Information

The authors did not receive any form of funding for this study.

Author Contribution

OO: Conceptualization, design, supervision, review and editing, and approval of final draft

AOA: Design, microbe isolation, analysis and review

BPA: Data collection, investigation, methodology and first draft

OOT: Data collection, analysis, review and editing.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors wish to acknowledge the Hematology Laboratory of the Department of Veterinary Medicine, University of Ibadan for technical assistance during analysis.

References

Abdel-Tawwab, M. & Abbass, F. E. (2017). Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: Growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, *48*, 30–312. https://doi.org/10.1111/jwas.12349

Abdel-Tawwab, M., Adeshina, I., Jenyo-Oni, A., Ajani, E. K., & Emikpe, B. O. (2018). Growth, physiological, antioxidants, and immune response of African Catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection. *Fish and Shellfish Immunology*, *78*, 346–354.

https://doi.org/10.1016/j.fsi.2018.04.057

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, *105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Ahmed, S. M., Vrushabendra Swamy, B.M., Gopkumar, P., Dhanapal, R., & Chandrashekara, V. M. (2005). Anti-Diabetic Activity of *Terminalia Catappa* Linn. Leaf Extracts in Alloxan-Induced Diabetic Rats*. Iranian Journal of Pharmacology and Therapeutics*, 4(1), 36-39. https://sid.ir/paper/297074/en

Ajani, E. K., Orisasona, O., Kareem, O. K, Osho, E. F, Adeyemo, A. O, Omitoyin, B.O, & Adekanmbi, A. O. (2020). Growth performance, gut ecology, immunocompetence and resistance of *Oreochromis niloticus* juveniles fed dietary *Curcumin longa*. *Croatian Journal of Fisheries*, 78(3):145-156. DOI: 10.2478/cjf-2020-0014Ajeel, S. G., & Al-Faragi, J. K. (2013). Effect of ginger, *Zingiber officinale* and garlic, *Allium sativum* to enhance health of common carp, Cyprinus carpio. *The Iraqi Journal of Veterinary Medicine*, *37*, 59–62. https://www.iraqoaj.net/iasj/article/78647

Akinwande, A., Ogunshakin, R & Krishnamurthy, R (2016). Non Specific Immune Response in the African Catfish, *Heterobranchus Longifilis* Fed Diets Fortified with Ethanolic Extracts of Selected Traditional Medicinal Plants and Disease Resistance against *Pseudomonas Aerugionsa. Journal of Agricultural Research and Development*, 15(1): 9-23.

Bae, J.Y., Lee, Y.S., Han, S.Y., Jeong, E.J., Lee, M.K., Kong J.Y., & Ahn, M.J. (2012). A comparison between water and ethanol extracts of Rumex acetosa for protective effects on gastric ulcers in mice. *Biomolecules & Therapeutics*., 20: 425-430. https://doi.org/10.4062/biomolther.2012.20.4.425.

Castell, J.D.& Tiews, K (1979). International Union of Nutritional Sciences and International Council for the Exploration of the Sea. 1980. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research: (Hamburg, Federal Republic of Germany, 21-23 March 1979). Food and Agriculture Organization of the United Nations, Hamburg, Germany. 24 pp.

Chakraborty, S.K., Horn, P., & Hancz, C., (2013). Application of phytochemicals as growth promoters and endocrine modulators in fish culture. *Reviews in Aquaculture 5: 1-19.* https://doi.org/10.1111/raq.12021

Chowdhury, S., Rheman, S., Debnath, N., Delamare-Debouteville, J., Akhtar, Z., Ghosh, S., Parveen, S., Islam, K., Islam, Md. A., Rashid, M., Khan, Z. H., Rahman, M., Chadag, V. M., & Chowdhury, F. (2022). Antibiotics usage practices in aquaculture in Bangladesh and their associated factors. *One Health 15, 100445*. https://doi.org/10.1016/j.onehlt.2022.100445

Culling, C. F. A. (1974). Handbook on histopathological and histochemical techniques: Including museum techniques, 3rd ed. Butterworth Heinemann, Oxford, UK. 726 pp.

Do, Q. D, Angkawijaya, A. E., Tran-Nguyen. P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S, *et al*. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*. 22:296–302. doi: 10.1016/j.jfda.2013.11.001Drury, R. A. B., Wallington, E. A., & Cameron, R. (1967). Carleton's histolog ical techniques (pp. 279–280). New York, NY: Oxford University Press.

El-Bahar, H. M., Ali, N. G., Aboyadak, I. M., Khalil, S. A. E. S & Ibrahim, M. S (2019). Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in *Oreochromis niloticus*. *International Microbiology*. 22(4):479-490. https://doi.org/10.1007/s10123-019-00075-3

Eyarafe, O.D., Emikpe, B. & Arowolo, O. (2008). Small bowel responses to enteral honey and glutamine administration following massive small bowel resection in rabbit. *African Journal of Medicine and Medical Sciences,* 37(4): 309-314. https://pubmed.ncbi.nlm.nih.gov/19301706/

Galvez, J., Duarte, J., & Sanchez, F. (1996). Inhibitory effects of quercetin on guinea pig ileum contractions. *Phytotherapy Research*, *10*, 66–69. https://doi.org/10.1002/(SICI)1099-1573(19960 2)10:1<66:AIDPTR778>3.0.CO;2-B.

Giacobbo, A., Do Prado, J. M., Meneguzzi, A., Bernardes, A. M. & de Pinho, M. N. (2015). Microfiltration for the recovery of polyphenols from winery effluents. *Separation and Purification Technology*. 143:12–8. https://doi.org/10.1016/j.seppur.2015.01.019

Gonfa, T., Teketle, S & Kiros, T. (2020). Effect of extraction solvent on qualitative andquantitative analysis of major phyto-constituentsand in-vitro antioxidant activity evaluation of Cadaba rotundifolia Forssk leaf extracts. *Cogent Food & Agriculture*, 6: 1853867 https://doi.org/10.1080/23311932.2020.1853867

Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*. 249(22):7130-7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Helland, S. J., Grisdale-Helland, B., & Nerland, S. (1996). A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture*, *139*, 157–163. https://doi.org/10.1016/0044-8486(95)01145-5

Ilavazhahan, M., Tamilselvi, R., & Sujatha, L. B. (2012). Biochemical alter ation in the muscle, liver, kidney and brain of a fresh water fish, Catla catla (Ham.) exposure of a heavy metal toxicant ferrous sulphate. *Biomedical & Pharmacology Journal*, 5(2), 261–272.

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P. & Khelurkar, V.C. (2017). Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemical 6:32-36.* https://www.phytojournal.com/archives/2017/vol6issue1/PartA/6-1-23-924.pdf

Irshath, A. A., Rajan, A. P., Vimal, S., Prabhakaran, V. & Ganesan, R. (2023)**.** Bacterial pathogenesis in various fish diseases: Recent advances and specific challenges in vaccine development. *Vaccines Basel, 11(2), 470*. https://doi.org/10.3390/vaccines11020470

Jain, C. N. (1986). *Schalm's veterinary haematology* (4th ed.). Philadelphia, PA: Lee and Febiger Publishing.

Kaplow, L. S. (1955). A histochemical procedure for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood*, 10(10): 1023-1029. https://doi.org/10.1182/blood.V10.10.1023.1023

Kocour, M., Lynhard, O., Gela, D., & Rodina, M. (2007). Growth performance of all–female and mixed–sex common carp, *Cyprinus carpio* L. population in central European

climatic conditions. *Journal of the World Aquaculture Society*, *36*, 103–113. https://doi.org/10.1111/J. 1749-7345.2005.TB00136.X

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *Biological Chemistry*, *193*, 265–275.

Lim, K.J.A., Cabajar, A.A., Lobarbio, C.F.Y., Taboada, E.B. & Lacks, D.J. (2019). Extraction of bioactive compounds from mango (Mangifera indica L. var. Carabao) seed kernel with ethanol–water binary solvent systems. *Journal of Food Science and Technology*. 56:2536–44. https://doi.org/10.1007/s13197-019-03732-7

Magnadottir, B., (2010). Immunological control of fish diseases. *Marine Biotechnology 12, 361-379.* https://doi.org/10.1007/s10126-010-9279-x.

Misra, H.P. and Fridovich, I. (1972) The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry*, 247: 3170-3175.

Misra, C. K., Das, B. K., Mukherjee, S. C., & Meher, P. K. (2006). The immunomodulatory effects of tuftsin on non-specific immune system of Indian major carp, *Labeo rohita*. *Fish and Shellfish Immunology*, *20*, 728–738. https ://doi.org/10.1016/j.fsi.2006.09.004

Mithöfer, A. & Maffei, M. E. (2017). General mechanisms of plant defense and plant toxins In: P Gopalakrishnakone, CR Carlini and R Ligabue-Braun, editors. Plant toxins. Dordrecht: Springer Netherlands. 3–24.

Omitoyin, B. O., Ajani, E. K., Orisasona, O., Bassey, H.E., Kareem, K. O., & Osho, F. E. (2019): Effect of guava *Psidium guajava* (L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Aquaculture Research*., 50, 1851–1861. https://doi.org/10.1111/are.14068

Plaskova, A. & Mlcek, J. (2023). New insights of the application of water or ethanol-water plant extract rich in active compounds in food. *Frontiers in Nutrition* 10:1118761. https://doi.org/10.3389/fnut.2023.1118761

Raj, N. S., Swaminathan, T. R., Dharmaratnam, A., Raja, S. A., Ramraj, D & Lal, K. K (2019). *Aeromonas veronii* caused bilateral exophthalmia and mass mortality in cultured Nile tilapia, *Oreochromis niloticus* (L.) in India. *Aquaculture*, 512, 734278. https://doi.org/10.1016/j.aquaculture.2019.734278

Reitman, S, & Frankel, S. (1957) A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *American Journal of Clinical Pathology* 28: 56-58. https://doi.org/10.1093/ajcp/28.1.56

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B. & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture Research 43(3):50-61*. https://doi.org/10.1016/j.aquaculture.2014.05.048.

Robert, E. A., Yisa, A. T & Tsadu, S. M. (2019). Growth performance and survival of monosex cultured Heterobranchus longifilis juveniles in concrete flow-through and stagnant water systems. *Scientific Research Journal* 2(2): 43-65. https://dx.doi.org/10.31364/SCIRJ/v7.i2.2019.P0219XX

Suleiman, A. M., Orire, A. M., Sadiku, S. O. E & Bake, G. G. (2023). Growth performance, nutrient utilization and survival rate of *Clarias gariepinus* fed varied inclusion of processed *Moringa oleifera* diets. *International Journal of Fisheries and Aquatic Studies* 11(1): 36-40. Doi: https://doi.org/10.22271/fish.2023.v11.i1a.2768

Sarwar, S., Anwar, F., Raziq, S., Nadeem, M., Zreen, Z. & Cecil, F. (2012). Antioxidant characteristics of different solvent extracts from almond (Prunus dulcis L.) shell. *Journal of Medicinal Plants Research* 6(17): 3311-3316. https://doi.org/10.5897/JMPR11.1723

Svobodová, Z., Pravda, D., Palácḱova, J., & Research Institute of Fish Culture and Hydrobiology (Vodnany, Czechoslovakia). (1991). *Unified methods of haematological examination of fish*. Research Institute of Fish Culture and Hydrobiology, Pp 31.

Tietz, N. W., Burtis, C. A., Duncan, P., Ervin, K., Petitclerc, C. J., Rinker, A. D., … Zygowicz, E. R. (1983). A reference method for measurement of alkaline phosphatase activity in human serum. *Clinical Chemistry*, *29*, 751–761.

Vankampen, E. J., & Ziglstra, W. G. (1961). Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clinica Chemica Acta*, *6*, 538-544. https://doi.org/10.1016/0009-8981(61)90145-0

Yadav, M., Chatterji, S., Gupta, S. K & Watal, G. (2014). Preliminary Phytochemical Screening of Six Medicinal Plants Used in Traditional Medicine. *International Journal of Pharmacy and Pharmaceutical Sciences.* 6(5): 539–542. https://www.innovareacademics.in/journal/ijpps/Vol6Issue5/9439.pdf