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Safety Evaluation of Ripe Banana Peel (*Musa acuminata*) Waste as a Potential Aquaculture Feed Additive through Acute Toxicity and Histopathological Studies in Juvenile *Clarias gariepinus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

The safe use of agricultural wastes in aquaculture feeds requires prior toxicological assessment. This study evaluated the acute toxicity and histopathological effects of an ethanolic extract of ripe banana peel (*Musa acuminata*) in juvenile African catfish (*Clarias gariepinus*). Fish were exposed for 96 h to 0, 20, 25, 30, or 35 mg/L of the extract in two experimental batches, with 10 juveniles per aquarium. Mortality, behavioural responses, water quality, and histological changes in the gills and liver were assessed. No mortality occurred in the control. In Batch A, no mortality occurred at 20 or 25 mg/L, whereas Batch B recorded 20% mortality at both concentrations. Mortality reached 50% at 30 mg/L and 60% at 35 mg/L in both batches, and the 96-h median lethal concentration was estimated at 30 mg/L. Behavioural abnormalities, including air gulping, erratic swimming, respiratory distress, and vertical erection, generally intensified with concentration and exposure duration. Significant differences were observed in dissolved oxygen, temperature, pH, and conductivity across treatments. Gill architecture remained largely preserved, although mild lamellar alteration occurred at 30 mg/L. Liver sections showed steatosis, hepatocellular vacuolation, peripheral nuclear displacement, and vascular congestion; however, severe necrosis and complete architectural disruption were not observed. The extract therefore produced measurable acute toxicity at higher concentrations and mild to moderate tissue alterations. Chronic feeding trials and additional biochemical and histopathological assessments are required before its use as an aquaculture feed additive.

Keywords: Clarias gariepinus; banana peel extract; acute toxicity; histopathology; feed additive.

1. Introduction

Aquaculture has emerged as one of the fastest-growing food-production sectors globally (FAO, 2024; Otoh et al., 2024a, 2024b) and plays a critical role in ensuring food security, generating employment, and supporting economic development (Otoh et al., 2024c, 2024d). The increasing demand for fish protein, coupled with declining yields from capture fisheries, has necessitated the expansion and intensification of aquaculture production systems (Inyang-Etoh et al., 2022; Otoh et al., 2022, 2023, 2025). Among cultured fish species in Africa, the African catfish (*Clarias gariepinus*) remains one of the most economically important because of its rapid growth, high tolerance of environmental fluctuations, efficient feed conversion, and strong consumer acceptance (Inyang-Etoh et al., 2022; Otoh et al., 2022, 2023, 2025). Consequently, the species has become a cornerstone of commercial aquaculture in Nigeria and many other African countries.

Despite the remarkable growth of the aquaculture industry, feed remains the most expensive component of fish production, accounting for approximately 70–80% of total operational costs (Tacon & Metian, 2015). The high cost of conventional feed ingredients, such as fishmeal, soybean meal, and maize, has significantly reduced profitability and limited the expansion of aquaculture enterprises, particularly among small-scale farmers. This challenge has stimulated considerable interest in exploring alternative, locally available, and cost-effective feed resources capable of partially replacing conventional ingredients without compromising fish health and performance.

Agricultural wastes and agro-industrial by-products have received increasing attention as sustainable alternatives in aquaculture feed formulation. The utilisation of these materials not only reduces feed costs but also contributes to environmental sustainability by minimising waste accumulation and pollution. Among such agricultural residues, banana peels represent a substantial proportion of fruit-processing waste generated worldwide (Serratos et al., 2025). Bananas are among the most widely cultivated and consumed fruits, and their peels constitute approximately 30–40% of the total fruit weight (Serratos et al., 2025). In many developing countries, including Nigeria, large quantities of banana peels are discarded indiscriminately into the environment, contributing to solid-waste management challenges and environmental degradation. Recent reviews have identified fruit and vegetable processing by-products as potentially valuable aquafeed ingredients, while emphasising the need for processing approaches that improve nutrient availability and ensure feed safety (Lourenço et al., 2025; Malik et al., 2025). Fruit peel wastes have likewise been considered low-cost resources that may support waste valorisation and more sustainable fish production (Thakur et al., 2025).

Ripe banana (*Musa acuminata*) peels contain several nutrients and bioactive compounds, including carbohydrates, dietary fibre, proteins, essential minerals, vitamins, and phytochemicals such as flavonoids,

tannins, phenolics, and antioxidants (Serratos et al., 2025). These nutritional and functional properties have stimulated interest in their potential application as livestock and aquaculture feed ingredients. Previous studies have demonstrated that banana peel meal may serve as a supplementary energy source and dietary additive in animal nutrition (Serratos et al., 2025). Furthermore, the presence of antioxidant compounds suggests that banana peels may possess health-promoting properties capable of enhancing growth, immunity, and resistance to oxidative stress in cultured organisms. A tilapia feeding trial also indicated that low-level supplementation with fruit wastes, including banana peel, may influence weight gain and feed conversion, supporting further evaluation of banana peel-derived materials in aquafeeds (Gonzales-Plasus et al., 2022).

However, despite their nutritional potential, banana peels also contain anti-nutritional factors and secondary metabolites whose effects on aquatic organisms remain incompletely understood. Compounds such as tannins, oxalates, phytates, saponins, and certain phenolic constituents may exert adverse physiological effects when present in excessive concentrations. The accumulation of these substances in formulated diets may impair nutrient utilisation, induce physiological stress, or cause tissue damage in fish. Accordingly, processing and mitigation strategies are commonly considered when plant-based aquafeed ingredients contain anti-nutritional factors that may limit nutrient utilisation or fish performance (Punia et al., 2024).

Therefore, before ripe banana peels can be recommended as feed additives or feed ingredients in aquaculture, their safety profile must be thoroughly evaluated. Toxicity studies provide valuable information on the potential hazards associated with novel feed resources. Acute toxicity tests are commonly used to determine the immediate effects of bioactive substances on fish survival and behaviour under controlled exposure conditions (Ojutiku et al., 2014; Ogbeide et al., 2019; Musa & Imam, 2022). Mortality patterns and behavioural responses are sensitive indicators of physiological stress and toxic effects (Erdoğan et al., 2005; Çapkin et al., 2010; George et al., 2023a, 2023b). Behavioural alterations, such as erratic swimming, loss of equilibrium, excessive mucus secretion, air gulping, and hyperactivity, often precede mortality and can provide early warning signs of adverse effects (Sula et al., 2020; George et al., 2025a, 2025b).

In addition to mortality and behavioural observations, histopathological assessment is recognised as one of the most reliable biomarkers for evaluating sublethal toxicity in fish (George et al., 2025a, 2025b). Histological examination enables the detection of structural alterations in target organs that may not be apparent through external observation alone (George et al., 2025c, 2025d). The gills and liver are particularly important organs in aquatic toxicology because they are among the first tissues to interact with environmental contaminants. The gills serve as the primary site for respiration, osmoregulation, and excretion, making them highly susceptible to toxic substances present in the aquatic environment (George et al., 2024a, 2024b). Similarly, the liver functions as the principal organ for metabolism, detoxification, and storage of nutrients and xenobiotics, rendering it vulnerable to chemically induced injury (George et al., 2024c, 2024d). Histopathological changes in these organs therefore provide valuable insights into the physiological effects of potentially toxic substances and the mechanisms underlying those effects.

The African catfish (*Clarias gariepinus*) is widely used as a model species in ecotoxicological and nutritional studies because of its ecological relevance, economic importance, and sensitivity to environmental stressors (Agboola & Fawole, 2014; Ojutiku et al., 2014). Evaluating the toxicological effects of ripe banana peel extracts on this species is therefore essential for determining the suitability of banana peel-derived products for aquaculture applications. Although several studies have investigated the nutritional composition and utilisation of banana peels in animal feeding, information on the acute toxicity and histopathological effects of ripe banana peel extracts on fish remains limited. Such information is necessary to establish safe inclusion levels and prevent unintended adverse effects on fish health and productivity.

Therefore, this study was undertaken to evaluate the safety of ripe banana peel waste as a potential aquaculture feed additive through acute toxicity testing and histopathological assessment in juvenile *Clarias gariepinus.*

2. Materials and Methods

2.1 Collection of Plant Sample

Fresh peels of ripe banana *(Musa acuminata)* were collected from Obio Akpa in Oruk Anam Local Government Area, Akwa Ibom State, on 29 August 2025. The samples were transported to the University of Uyo, Uyo,

Akwa Ibom State, for plant identification and authentication at the Herbarium of the Department of Botany and Ecological Studies.



Fig. 1. Fresh ripe banana peels (*M. acuminata)* used in the study (Source: Authors Experimental Preparation, 2026)

2.2 Preparation of Plant Material

After identification, the peels of *M. acuminata* were washed, cut into smaller pieces, and briefly spread under the sun to drain surface water. They were then placed on cellophane and dried for 14 days at room temperature. The dried peels were pulverised into a fine powder using a wooden pestle and mortar.



Fig. 2. Spreading of the ripe banana peels (*M. acuminata)* under the sun used for the study. (Source: Authors Experimental Preparation, 2026)



Fig. 3. Dried ripe banana peels (*M. acuminata)* used in the Study. (Source: Authors Experimental Preparation, 2026)

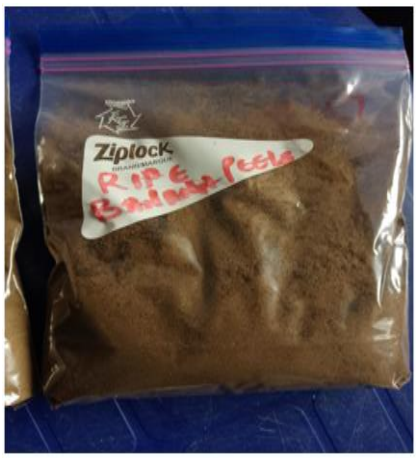


Fig. 4. Powdered ripe banana peels *(M. acuminata)* used in the study. (Source: Authors Experimental Preparation, 2026)

2.3 Preparation of Ethanolic Extract (Maceration and Extraction)

The cold extraction method (maceration) was used following Hidayat and Wulandari (2021). In this process, 240 g of plant material was macerated in 1,000 mL of 99% ethanol in an airtight container and kept at room temperature for 72 h (3 days). The ethanolic suspension was subjected to filtration using a filter net followed by filter paper, after which the filtrate was concentrated by evaporation in a water bath maintained at 40 °C for 48 h. The resulting extract was stored in a beaker covered with aluminium foil and was subsequently utilised for the bioassay immediately upon completion of the evaporation process.

2.4 Collection of Test Organism

Juveniles of Clarias gariepinus were obtained from the Akwa Ibom State University fish farm, Obio Akpa, Akwa Ibom State, Nigeria. The fish were acclimatised for 24 h in a recirculating plastic aquarium (25 × 13 × 8.3 cm^3) containing hatchery water within the Fisheries and Aquaculture Laboratory of the fish farm. This acclimatisation period helped to stabilise the fish and minimise stress resulting from collection and transportation (George et al., 2015a, 2015b).



Fig. 5. Juveniles of *Clarias gariepinus* used in the study. (Source: Authors Experimental Preparation, 2026)

2.5 Preliminary Test

Before the actual experiment commenced, a preliminary range-finding test was conducted at concentrations of 0, 5, 10, 15, and 20 mg/L to determine appropriate concentration levels for the bioassay. Each aquarium had a

replicate to improve the reliability of the results. Each of the 10 plastic aquaria was filled with 2 L of hatchery water, and 10 *Clarias gariepinus* juveniles were stocked in each aquarium. The ethanolic extract of *M. acuminata* was added at concentrations of 0, 20, 25, 30, and 35 mg/L, and the experimental set-up was maintained for 96 h for mortality assessment (George et al., 2013a, 2013b, 2014).

2.6 Monitoring of Water Quality

Water quality parameters were assessed prior to the commencement of the experiment and monitored periodically throughout the study in accordance with standard procedures (APHA, 2005). The parameters evaluated included dissolved oxygen (DO), pH, temperature (°C), and conductivity. Temperature, pH, and conductivity were determined using a portable pH/EC/TDS/temperature meter (HANNA H1 991301), while dissolved oxygen levels were measured using a digital portable analyser (JPB-607A, Search Tech Instrument).

2.7 Determination of Mortality and Survival Rates of Juveniles

The percentage mortality and survival rates of the juveniles exposed to different concentrations of the ethanolic extract of *M. acuminata* during the study period were determined using the following formula:

$$\% \text{ mortality} = n/N \times 100 \text{ (Chan, 1977)}$$

where:
n = number of dead fish per aquarium at each concentration
N = total number of individuals stocked

The percentage survival of the juveniles at the end of the 96-h experiment was calculated from the difference between the number stocked and the number that died (George et al., 2023c, 2023d).

2.7.1 Determination of the 96-h Median Lethal Concentration (LC_{50})

The effects of different concentrations of the ethanolic extract of M. acuminata on juvenile C. gariepinus were evaluated using the graphical probit analysis method described by Finney (1971). The 96-h median lethal concentration (LC_{50}) was estimated from the graph as the concentration expected to cause mortality in 50% of the juveniles. Similarly, the concentration corresponding to 50% mortality at the end of the 96-h test was determined at the probit level.

2.7.2 Behavioural and Morphological Measurements

Behavioural and morphological responses of C. gariepinus juveniles exposed to the ethanolic extract of M. acuminata were monitored daily in accordance with OECD (1992) guidelines. Control groups, which were not exposed to the toxicant, were similarly observed to provide a reference for evaluating treatment-related behavioural and morphological alterations. The observed responses included erratic swimming, excessive mucus production, increased air gulping, vertical erection, respiratory impairment, and changes in skin pigmentation. All experimental tanks were monitored throughout the exposure period.

2.7.3 Collection of Samples for Histopathological Examinations

The gill and liver tissues were excised from the experimental animals and fixed in formalin-saline solution for 48 h. The fixed tissues were subsequently processed manually through a series of graded ethanol concentrations, cleared in xylene, impregnated, and embedded in paraffin wax. Tissue sections were prepared using a rotary microtome and stained with the haematoxylin and eosin technique. The stained sections were then examined microscopically at ×100 and ×400 magnifications to assess histopathological alterations.

2.8 Data Analysis

The effects of the different concentrations of the ethanolic extract of M. acuminata were presented in tabular form. One-way analysis of variance (ANOVA) was employed to determine significant differences among the

various concentrations in both batches (A and B), with statistical significance set at $p < 0.05$. Probit analysis was conducted using SPSS software version 20.0.

3. Results

3.1 Initial Water Quality Parameters

The initial water quality parameters before the toxicity study are shown in Table 1. The observed values for dissolved oxygen (4.2 mg/L), temperature (28.0 ˚C), pH (5.58), and conductivity (0.05 µS) were within the acceptable range for aquaculture operations.

Table 1. Initial Physico-chemical parameters of the test water prior to stocking of test organism

Fish Species	DO (mg/l)	Temp (°C)	pH	Conductivity (µS)
Clarias gariepinus (Juveniles)	4.2 mg/l	28.0 ^{0}C	5.58	0.05 µS

3.2 Variation in Physicochemical Parameters of the Test Media Containing *Clarias gariepinus* Juveniles during the 96-h Experimental Period

Variations in the water quality parameters (DO, temperature, pH, and conductivity) of the test media at different concentrations and exposure times are presented in Figs. 6–9.

3.2.1 Dissolved Oxygen (mg/L)

The lowest dissolved oxygen concentration was observed at 20 mg/L after 72 h, whereas the highest DO level was observed in the control (0 mg/L) after 24 h. Variations in DO at different time intervals and concentrations are shown in Fig. 6. At 24, 48, 72, and 96 h, significant variations ($p < 0.05$) were observed among the extract concentrations.

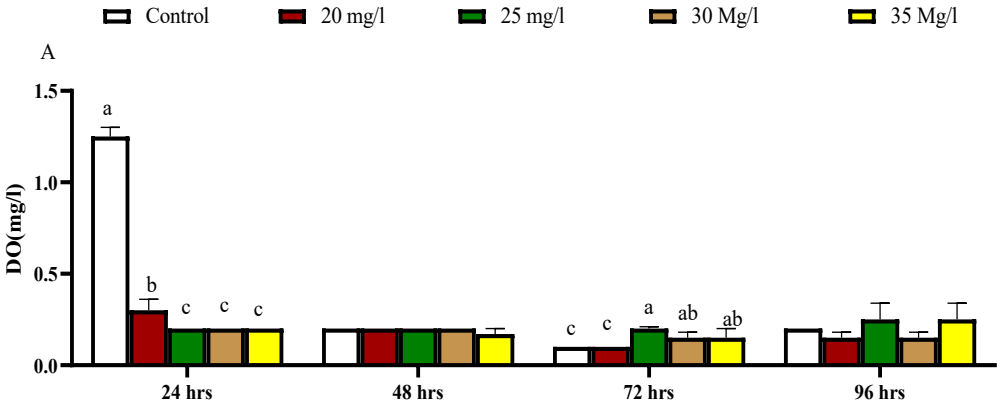


Fig. 6. Dissolved oxygen variations during the 96 Hours of Test (Ripe banana peels ethanolic extract)

3.2.2 Temperature (˚C)

For the ripe banana peel extract, significant variation ($p < 0.05$) in temperature was observed across the different concentrations, although the differences during the 96-h bioassay were small (Fig. 7).

3.2.3 pH

The pH varied during the 96-h experimental period. Significant variations ($p < 0.05$) were observed among all concentrations at the different time intervals (Fig. 8). However, pH values were generally higher in the control tank across the different time intervals.

3.2.4 Conductivity

Conductivity values showed an interesting pattern, with high values recorded in the control tank at the different time intervals. However, significant variations ($p < 0.05$) were observed among all concentrations at 24, 48, 72, and 96 h (Fig. 9).

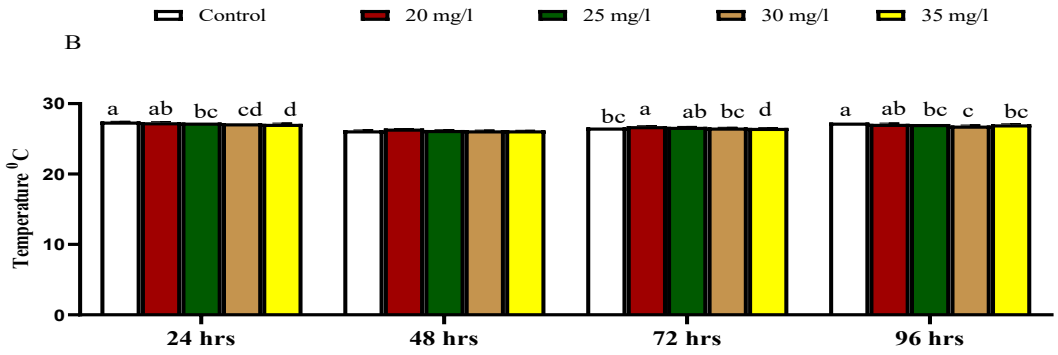


Fig 7. Temperature Variations during the 96 Hours of Test (Ripe Banana Peels Ethanolic Extract)

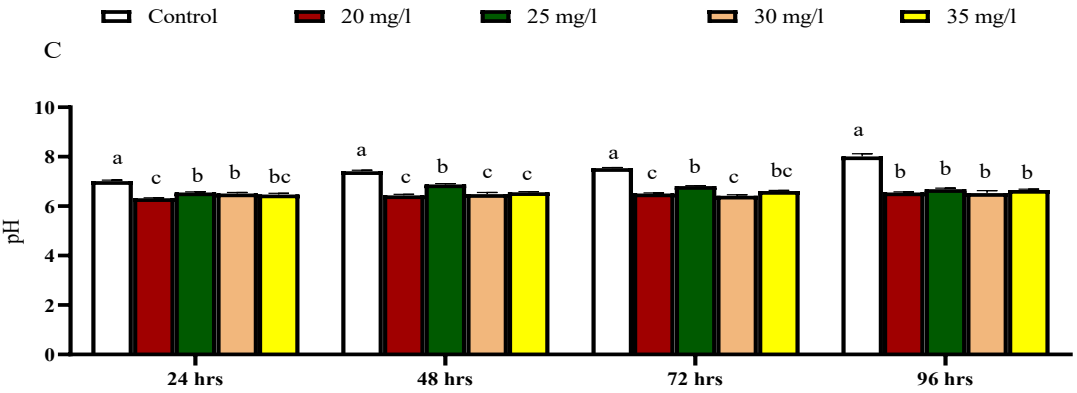


Fig 8. pH Variations during the 96 Hours of Test (Ripe Banana Peels Ethanolic Extract)

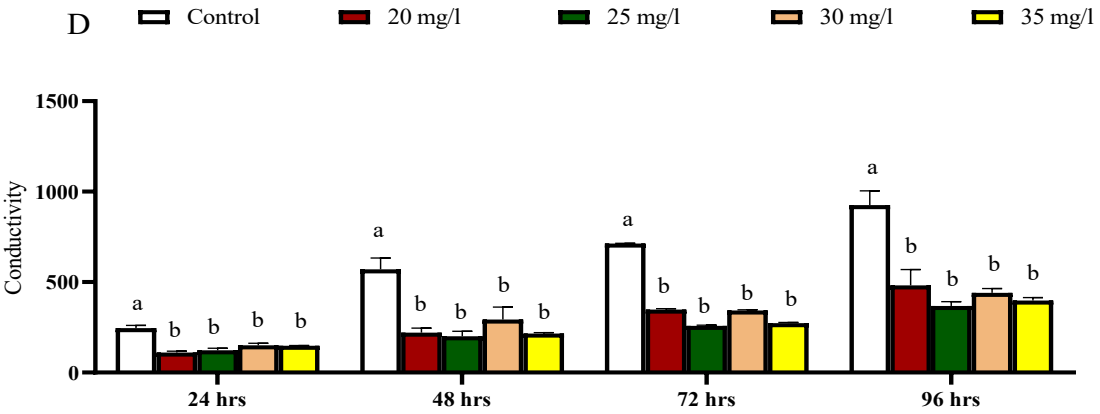


Fig 9. Conductivity Variations during the 96 Hours of Test (Ripe Banana Peels Ethanolic Extract)

3.3 Percentage Mortality and Survival of *C. gariepinus* Juveniles at Different Concentrations of the Ethanolic Extract of Ripe *M. acuminata* Peels after 96 h (Preliminary Test)

During the preliminary toxicity test of the different concentrations of the ethanolic extract of ripe *Musa acuminata* peels, no mortality was recorded in the control (0 mg/L), resulting in 100% survival in both Batches A and B during the 96-h exposure period. Similar results were observed at 5, 10, 15, and 20 mg/L in both batches during the 96-h exposure period.

3.4 Summary of Percentage Mortality and Survival of *C. gariepinus* Juveniles at Different Concentrations of the Ethanolic Extract of Ripe *M. acuminata* Peels after 96 h

The percentage mortality and survival of *Clarias gariepinus* juveniles exposed to the different concentrations of the ethanolic extract of ripe *M. acuminata* peels are presented in Table 2.

No mortality was recorded in the control (0 mg/L) during the 96-h bioassay in either batch. At 20 and 25 mg/L, no mortality was recorded in Batch A; however, a marked deviation was observed in Batch B, in which 20% mortality occurred at both concentrations. At 30 mg/L, 50% mortality was recorded in both Batches A and B, leaving 50% survival in each batch.

At 35 mg/L, 60% mortality was recorded in both Batches A and B, leaving 40% survival in each batch.

Table 2. Summary of the Percentage Mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanolic extract of ripe peels of *M. acuminata* at the end of the experiment (96 hours)

Conc. of extract (mg/l)	BATCH A				BATCH B			
	Mortality (M)	% M	Survivors (S)	% S	Mortality (M)	% M	Survivors (S)	% S
0 mg/l	0	0	10	100	0	0	10	100
20 mg/l	0	0	10	100	2	20	8	80
25 mg/l	0	0	10	100	2	20	8	80
30 mg/l	5	50	5	50	5	50	5	50
35 mg/l	6	60	4	40	6	60	4	40

3.5 Determination of the 96-h LC_{50} for Ripe *M. acuminata Peels*

The 96-h median lethal concentration (LC_{50}) for C. gariepinus juveniles exposed to varying concentrations of the ethanolic extract of ripe M. acuminata peels was estimated using probit analysis. Prior to analysis, the exposure concentrations were subjected to logarithmic transformation (Table 3). The estimated 96-h LC_{50} value was 30 mg/L, corresponding to a log-transformed concentration of 1.47, indicating the concentration at which 50% of the exposed organisms were predicted to experience mortality by the end of the experimental period.

Table 3. LC_{50} determination for *C. gariepinus* Juveniles at the end of the 96-hours bioassay for ripe Banana (*M. acuminata)* Peels extract

Concentration (mg/l)	Log Transformation	Mortality (M)	% Mortality	Survivors (S)	% Survivor
0 mg/l	0	0	0	10	100
20 mg/l	1.30	2	20	8	80
25 mg/l	1.39	2	20	8	80
30 mg/l	1.47	5	50	5	50
35 mg/l	1.54	6	60	4	40

3.6 Behavioural Responses of *Clarias gariepinus* Juveniles to the Toxic Effects of Ethanolic Extract of Ripe *M. acuminata* Peels during a 96-h Bioassay

Behavioural responses of C. gariepinus juveniles exposed to the ethanolic extract of ripe M. acuminata peels for 24–96 h were evaluated using a semi-quantitative scoring approach (Table 4). Following 24 h of exposure, fish groups treated with concentrations ranging from 20 to 35 mg/L exhibited mild to moderate behavioural alterations, including air gulping, erratic swimming, respiratory distress, and vertical erection, while no such responses were observed in the control group (0 mg/L). Prolonged exposure (48–96 h) resulted in an intensification of these behavioural abnormalities in a concentration-dependent manner, with the exception of the responses recorded at 20 and 25 mg/L.

Table 4. Effect behavioral responses of *Clarias gariepins* Juveniles to the toxic effects of ethanolic extract of peels of ripe *Musa acuminata* for 96 hours bioassay at different time intervals

Time in hours	Conc. (mg/l)	Gulping of air	Vertical erection	Respiratory distress	Erratic swimming
24	control	-	-	-	-
	20	+	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	++	++	++	++
48	control	-	-	-	-
	20	+	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	++	++	++	++
72	control	-	-	-	-
	20	-	-	-	-
	25	+	+	+	+
	30	++	++	++	++
	35	+++	+++	+++	+++
96	control	-	-	-	-
	20	-	-	-	-
	25	+	+	+	+
	30	+++	+++	+++	+++
	35	+++	+++	+++	+++

Where; - = Normal + = Mild ++ = Moderate +++ = Severe

3.7 Histopathology of the Gills and Liver of *C. gariepinus* Juveniles Exposed to Different Concentrations of the Ethanolic Extract of Ripe *Musa acuminata* Peels after 96 h

3.7.1 Gill Histopathology

Gill architecture remained largely intact across all treatment groups. Control fish (0 mg/L) displayed normal primary lamellae with orderly secondary lamellae, intact epithelium, and preserved vascular structures. Fish exposed to 20, 25, and 35 mg/L showed histological features comparable to those of the control, with well-preserved lamellar organisation and no pathological alterations. The single exception was the 30 mg/L group, which exhibited mild structural modifications of the lamellae; however, the overall gill architecture was maintained, with no evidence of epithelial degeneration, necrosis, or lamellar fusion.

3.7.2 Liver Histopathology

Liver sections from all groups, including the control, displayed hepatocellular vacuolation and steatosis. Control fish (0 mg/L) showed diffuse steatosis with widespread lipid-laden vacuoles and peripheral nuclear displacement. Fish exposed to 20 mg/L exhibited the most severe changes: diffuse macrovesicular steatosis with extensive vacuolation and marked nuclear peripheralisation. Both the 25 and 35 mg/L groups showed moderate,

diffuse vacuolation with peripheral nuclear displacement, without the severity observed at 20 mg/L. The 30 mg/L group displayed diffuse steatosis with a vacuolar pattern comparable to that of the control, but also showed dilated, congested blood vessels that were not present in any other group.

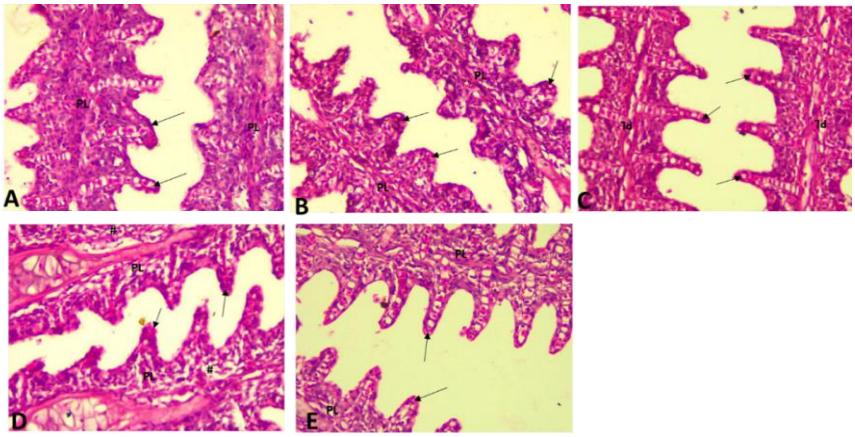


Fig. 10. Photomicrographs of the gills of *Clarias gariepinus* exposed to varying concentrations of *Musa paradisiaca* (banana peel) extract. (A–E) Gills Histopathology. (A) Control group (0 mg/L) showing normal primary lamellae (PL) with orderly secondary lamellae (thin arrow) and intact vascular structures. (B, C, E) Fish exposed to 20 mg/L, 25 mg/L, and 35 mg/L, respectively, displaying well-preserved lamellar organization and intact architecture comparable to the control. (D) 30 mg/L group showing mild structural modifications of the lamellae, though the overall architecture remained maintained without evidence of necrosis or lamellar fusion

The severity of hepatic steatosis did not follow a linear dose–response relationship. The 20 mg/L concentration produced the most pronounced hepatocellular damage, whereas the intermediate and high concentrations showed moderate, comparable changes. Vascular congestion appeared exclusively at 30 mg/L, the same concentration that produced the only observable gill alterations. The presence of diffuse steatosis in control fish indicates pre-existing hepatic lipid accumulation unrelated to extract exposure, complicating the direct attribution of vacuolar changes to treatment effects.

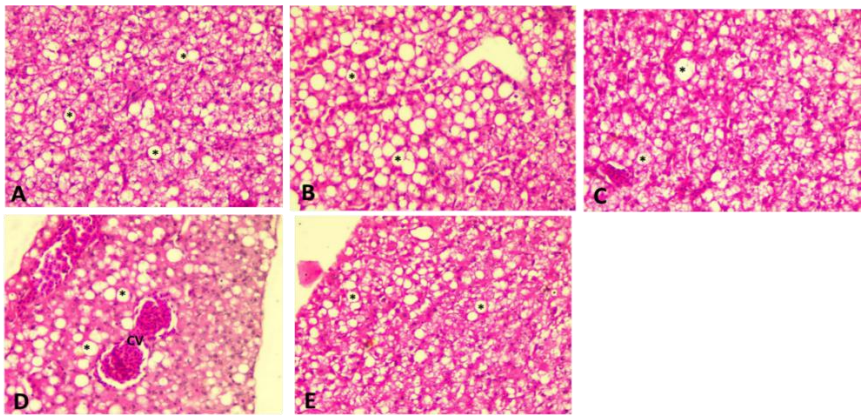


Fig. 11. Photomicrographs of the Liver of *Clarias gariepinus* exposed to varying concentrations of *Musa paradisiaca* (banana peel) extract. (A–E) Liver Histopathology. (A) Control group (0 mg/L) displaying diffuse hepatocellular vacuolation and steatosis with widespread lipid-laden vacuoles and peripheral nuclear displacement. (B) 20 mg/L group exhibiting peak macrovesicular steatosis (asterisk) characterized by extensive vacuolation and marked nuclear peripheralization. (C, D) 25 mg/L and 35 mg/L groups showing moderate, diffuse vacuolation and peripheral nuclear displacement. (E) 30 mg/L group showing diffuse steatosis comparable to the control, distinguished by the presence of unique dilated and congested blood vessels (CV) not observed in other treatment groups

4. Discussion

The present study evaluated the acute toxicity of ripe banana peel extract in juvenile *Clarias gariepinus* through mortality, water quality alterations, and histopathological responses of the gills and liver. The findings demonstrated that the effects of the extract were concentration- and time-dependent, with increasing mortality and physiological stress at higher concentrations and longer exposure periods (Ayoola, 2008; Sula et al., 2020; Omitoyin et al., 1999; Essien-Ibok et al., 2024a, 2024b; Ezekiel et al., 2025).

The mortality pattern recorded in this study suggests that the extract possesses mild to moderate toxicity to *juvenile C. gariepinus*. No mortality was observed in the control or at 20 and 25 mg/L in Batch A, indicating that these concentrations were generally tolerated by the fish. However, the occurrence of 20% mortality in Batch B at the same concentrations suggests variability in the phytochemical composition or potency of the extract between batches (Ojogu et al., 2017; Geetha et al., 2019; George et al., 2024c, 2024d).

At higher concentrations (30 and 35 mg/L), mortality increased to 50% and 60%, respectively, confirming a dose-dependent toxic response. Similar concentration-dependent mortality patterns have been reported in fish exposed to botanical extracts. For instance, Ayotunde et al. (2010) reported increased mortality in *Clarias gariepinus* exposed to *Carica papaya* seed powder, whereas Fafioye et al. (2004) observed dose-related mortality in fish exposed to extracts of *Parkia biglobosa* and *Raphia vinifera*. Such responses are often attributed to bioactive phytochemicals, including tannins, saponins, alkaloids, flavonoids, and phenolic compounds, which can interfere with normal physiological processes.

The significant variations observed in dissolved oxygen, temperature, pH, and conductivity further suggest that the extract influenced the aquatic environment. Changes in water quality parameters during toxicity studies may arise from the decomposition of organic compounds present in plant materials and from metabolic wastes released by stressed fish. Reduced dissolved oxygen concentrations can increase physiological stress and impair respiration, thereby enhancing toxic effects. Similar observations were reported by Neuwinger (2004), who noted that plant-derived organic substances altered water chemistry and affected fish performance. The concentration-dependent changes observed in this study indicate that increasing extract concentrations may have contributed to environmental stress in addition to direct toxic effects.

Gill architecture remained intact across nearly all treatment groups, a finding that contrasts with the severe branchial lesions, epithelial lifting, lamellar disorganisation, haemorrhage, and necrosis reported in *C. gariepinus* exposed to other plant-derived substances at comparable or lower sublethal ranges (Ekpenyong et al., 2022; Hassan et al., 2023). Gills serve as the primary interface between the organism and its aquatic environment, and their histopathological status is widely regarded as a sensitive early indicator of waterborne toxicant exposure (Bernet et al., 1999; Strzyżewska et al., 2016). The preservation of normal lamellar epithelium, vascular structures, and secondary lamellar organisation across four of the five concentrations tested suggests minimal direct branchial toxicity within the acute exposure range examined. The mild structural alteration observed exclusively at 30 mg/L, the same concentration that produced hepatic vascular congestion, may indicate a systemic threshold effect at this dose rather than organ-specific sensitivity. Taken together, these findings suggest that ripe *M. acuminata* peel extract caused comparatively limited branchial injury in *C. gariepinus* under the present acute exposure conditions. However, the confounded hepatic baseline underscores the need for subchronic feeding trials with standardised basal diets and quantitative histopathological scoring before a definitive safety profile can be established.

The diffuse hepatic steatosis observed across all groups, including the controls, warrants careful interpretation. Hepatocellular lipid accumulation in *C. gariepinus* is a recognised consequence of dietary lipid composition and feeding level rather than toxicant exposure alone. Verreth et al. (1994) demonstrated that *C. gariepinus* larvae fed at optimal or supra-optimal levels accumulated lipid exceeding 40% of hepatocyte volume, and Yılmaz and Genç (2006) documented hepatic lipidosis in fingerlings fed diets containing as little as 7% crude lipid. The baseline steatosis in the present study therefore likely reflects the lipid profile of the commercial feed used, which complicates attribution of the vacuolar changes to the banana peel extract. Against this confounded baseline, the non-monotonic severity pattern, with peak damage at 20 mg/L rather than at higher concentrations, is notable. Non-monotonic dose–response curves occur in an estimated 12–24% of toxicological studies (Lagarde et al., 2015) and are consistent with hormetic phenomena, in which low concentrations elicit greater biological perturbation than higher concentrations through adaptive or compensatory mechanisms (Calabrese &

Mattson, 2017; Wan et al., 2024). Alternatively, the pattern may reflect individual biological variability amplified by the small sample size. The vascular congestion observed exclusively at 30 mg/L parallels findings by Hassan et al. (2023), who reported sinusoidal congestion and vacuolation in *the liver of C. gariepinus* at sublethal concentrations of *Melaleuca cajuputi* extract, and by Ekpenyong et al. (2022), who documented vacuolar degeneration and hepatocyte necrosis in *C. gariepinus* exposed to *Justicia extensa*. The restriction of this lesion to a single concentration, however, further argues against a simple dose-dependent toxicity model.

Despite the observed hepatic alterations, severe degenerative lesions such as widespread necrosis, fibrosis, cellular disintegration, or complete disruption of hepatic architecture were not recorded. The preservation of overall tissue organisation suggests that the liver retained substantial structural integrity despite exposure. Severe hepatotoxicity is typically characterised by irreversible tissue destruction. The predominance of vacuolation and steatosis without extensive necrosis therefore suggests physiological stress rather than extensive organ injury.

The disparity between mortality responses and histopathological findings is noteworthy. Although mortality increased at higher concentrations, the gill and liver tissues generally maintained their structural integrity. This observation suggests that mortality may have resulted from a combination of physiological stress, water quality alterations, and systemic biochemical disturbances rather than direct, severe tissue destruction. Furthermore, the inability of even the highest concentration (35 mg/L) to produce 100% mortality after 96 h supports the view that the extract possesses relatively low acute toxicity to *juvenile C. gariepinus*.

Overall, the results indicate that the plant extract caused concentration-dependent mortality and induced mild to moderate histopathological changes in the liver while producing minimal alterations in the gills. The preservation of organ architecture and the absence of severe lesions suggest lower toxicity at the lower concentrations tested, but do not establish long-term safety. Further studies involving chronic exposure, biochemical biomarkers, oxidative stress parameters, and growth-performance assessments are required to establish long-term safety and efficacy.

5. Conclusion

This study demonstrated that ethanolic ripe *Musa acuminata* peel extract produced concentration- and time-dependent responses in juvenile *Clarias gariepinus*. The control group showed no mortality, whereas mortality increased to 50% at 30 mg/L and 60% at 35 mg/L after 96 h. Responses at 20 and 25 mg/L varied between batches, indicating that these lower concentrations were better tolerated but were not uniformly without adverse effects. Behavioural signs of stress became more pronounced with increasing concentration and exposure duration. Histological examination showed that gill architecture was generally maintained, with mild lamellar alteration only at 30 mg/L. The liver exhibited steatosis, vacuolation, peripheral nuclear displacement, and vascular congestion, but severe necrosis or complete tissue disruption was not observed. These findings indicate measurable acute toxicity at the higher exposure concentrations and comparatively limited structural injury during the 96-h test. The results do not establish long-term dietary safety. Further chronic feeding studies using standardised diets, broader physiological and biochemical endpoints, quantitative histopathological scoring, and growth-performance assessment are required before ripe banana peel-derived materials can be recommended as aquaculture feed additives.

6. Limitations

The study was limited to a 96-h acute exposure and two replicate batches, with a relatively small number of fish per treatment. Hepatic steatosis was also present in the control group, which complicated attribution of some liver alterations to the extract. The extract was administered through water rather than as a formulated dietary ingredient, so the findings do not directly represent long-term feed inclusion. Biochemical biomarkers, oxidative-stress indices, residue analysis, quantitative lesion scoring, growth performance, and chronic or subchronic outcomes were not assessed.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s)

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Competing Interests

Authors have declared that no competing interests exist.

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