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From agricultural waste to aquaculture feed: Acute toxicity, behavioural responses, water quality alterations and histopathological effects of ethanolic extract of unripe banana peels (*Musa acuminata*) on Juvenile *Clarias gariepinus*

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Abstract
This study evaluated the acute toxicity of ethanolic extract of unripe banana peels (*Musa acuminata*) on the survival, behaviour, water quality parameters, and histopathology of the gills and liver of juvenile African catfish, *Clarias gariepinus*. Juveniles were exposed for 96 hours to five concentrations of the extract: 0 (control), 20, 25, 30, and 35 mg/L under laboratory conditions. Water quality parameters (dissolved oxygen, pH, temperature, and conductivity) were monitored using standard analytical procedures, while histopathological examinations of gill and liver tissues were conducted following established histological techniques. No mortality was recorded in the control and 20 mg/L treatments, resulting in 100% survival. At 25 mg/L, 20% mortality was observed, leaving 80% survival after 96 hours. Mortality increased with concentration, reaching 50% and 60% at 30 and 35 mg/L, respectively, indicating a concentration-dependent toxic response. Behavioural abnormalities, including erratic swimming, frequent surfacing, gulping of air, and attempts to jump out of the exposure tanks, were more pronounced at higher concentrations. Significant variations ($p < 0.05$) were observed in dissolved oxygen, pH, temperature, and conductivity across treatment concentrations and exposure periods, suggesting deterioration of water quality with increasing extract concentration. Histopathological assessment revealed mild to moderate pathological alterations in the gills and liver, including tissue distortions and degenerative changes, with severity increasing alongside extract concentration. The findings demonstrate that ethanolic extract of unripe banana peels possesses toxic properties capable of inducing mortality, behavioural stress responses, histopathological lesions, and alterations in water quality parameters in juvenile *Clarias gariepinus*, highlighting potential ecological risks associated with its release into aquatic environments.

Keywords: *Musa acuminata*, *Clarias gariepinus*, agricultural waste valorization, aquaculture feed additive, acute toxicity, histopathology, water quality, sustainable aquaculture

1. Introduction
Aquaculture, the cultivation of aquatic organisms, stands as a cornerstone of global food security and economic development, particularly in developing nations like Nigeria (Otoh *et. al*., 2024a; b) [58, 59]. The African catfish (*Clarias gariepinus*) is a dominant species in Nigerian aquaculture due to its rapid growth rate, high market demand, resilience, and adaptability to diverse culture systems (Otoh *et. al*., 2024c; d) [61, 62]. Its cultivation contributes significantly to protein supply, job creation, and rural livelihoods across the country (Otoh *et. al*., 2022; 2023) [63, 64].
However, the sustained growth and profitability of the Nigerian aquaculture sector are severely hampered by a critical economic bottleneck: the prohibitive cost of fish feed. Feed typically constitutes the single largest operational expense, accounting for an estimated 60% to 80% of the total production cost in intensive and semi-intensive fish farming systems (Inyang-Etoh *et. al*., 2022; Otoh *et al*., 2025) [48, 60]. This exorbitant cost is primarily driven by the heavy reliance on expensive, often imported, conventional feed ingredients such as fishmeal and soybean meal. Other contributing factors include fluctuating exchange rates, high import duties, inflation, and

Inadequate local production capacity for high-quality feed components (Otoh *et al.*, 2022) [63]. The consequence is reduced profit margins for farmers, decreased fish production, and ultimately, higher consumer prices, which collectively undermine the industry's potential to address food insecurity.

Global aquaculture production continues to expand at an unprecedented rate, necessitating a proportional increase in the demand for high-quality, sustainable feed ingredients (Aisyah, *et al.*, 2021) [7]. As the industry seeks to minimize environmental impact and lower production costs, the search for sustainable, low-cost alternatives to traditional fish meal has turned attention toward agricultural by-products, with banana (*Musa spp.*) peels emerging as a promising candidate. While banana peels are typically discarded, they represent a "nutritious gold mine" rich in dietary fiber, essential minerals like potassium and magnesium, and bioactive compounds including flavonoids and tannins (Emaga, *et al.*, (2007) [25].

Considering this pressing challenge, there is an urgent and growing need to identify and develop cost-effective, locally available, and sustainable alternative feed ingredients and additives. The valorization of agricultural by-products, often discarded as waste, presents a promising avenue. Banana (Musa spp.), a major staple and cash crop in Nigeria, generates substantial quantities of banana peels as agricultural waste. These peels, constituting approximately 30-40% of the total fruit weight, are often underutilized, leading to environmental disposal issues (Anhwange *et al.*, 2009; Emelike and Obunadike, 2015) [11, 23].

Recent research has highlighted the potential of banana peels as a valuable resource due to their rich proximate composition and diverse biochemical profile. Proximate analysis reveals significant levels of carbohydrates, crude fiber, and even crude protein, along with essential minerals (Emelike and Obunadike, 2015) [23]. Furthermore, banana peels are abundant in various bioactive compounds, including phenolic compounds, flavonoids, tannins, saponins, and alkaloids (Ehiowemwenguan *et al.*, 2014; Zaini, *et al.*, 2020) [20, 68]. These compounds are known for their antioxidant, antimicrobial, and anti-inflammatory properties, suggesting their potential as functional feed additives to enhance fish growth, immunity, and overall health (Giri *et al.*, 2016) [45]. Indeed, studies have shown that the inclusion of banana peel flour in fish diets can improve growth performance and feed conversion ratios in species like common carp and rohu (Giri *et al.*, 2016) [45].

However, the efficacy and safety of using banana peels, particularly their extracts, as feed additives are critically dependent on their specific biochemical composition, which varies significantly with the stage of ripeness. Unripe banana peels are known to contain higher concentrations of certain anti-nutritional factors, such as tannins, which can impair nutrient digestibility and potentially exert toxic effects on aquatic organisms if not properly processed (Lawal *et al.*, 2014) [53]. Conversely, ripening processes lead to profound biochemical transformations, altering the carbohydrate profile (starch converting to simple sugars) and modifying the levels and types of various phytochemicals (Ayo *et al.*, 2022) [14]. These differences in chemical constituents between ripe and unripe peels suggest that they may possess distinct nutritional and toxicological profiles when considered as potential feed ingredients or additives.

While the nutritional benefits of incorporating banana peels into aquafeeds are being explored, a comprehensive understanding of their safety, particularly when concentrated extracts are considered for use as functional feed additives, is paramount. Fish, being aquatic organisms, are highly susceptible to waterborne and dietary toxicants. Their physiological responses, especially in vital organs like the gills and liver, serve as sensitive biomarkers of environmental stress and exposure (Bernet *et al.*, 1999) [16, 17]. The gills, constantly in contact with the external environment, are crucial for respiration and osmoregulation, making them primary targets for toxicants (Erdoğan, *et al.*, 2022, George *et al.*, 2023 a; b) [32, 40,] and are sensitive to even minor chemical or physical changes in the surroundings and are the target organ for many contaminants in water (Sula, *et al.*, 2020, George *et al.*, 2025a; b) [29, 30, 65]. The liver, as the central organ for metabolism and detoxification, is also highly vulnerable to chemical toxicity. Histopathological alterations in these organs, such as lamellar fusion, hyperplasia, and epithelial lifting in gills, or vacuolation, necrosis, and congestion in the liver, are direct indicators of cellular damage and impaired organ function (Abubakar *et al.*, 2023) [1]. Therefore, a detailed histopathological assessment of the effect of unripe banana peels is essential to determine the safety thresholds and potential adverse effects of this extracts.

2. Materials and Methods

2.1 Collection of plant sample

Fresh peels of unripe banana *(Musa acuminata)* were collected from Obio Akpa in Oruk Anam Local Government Area, Akwa Ibom State. The date of collection was on the 29th of August 2025. The banana was bought and transported to University of Uyo, Uyo, Akwa Ibom State for identification and authentication of the species. This was done at the Herbarium in the Department of Botany and Ecological studies, University of Uyo, Uyo.



Fig 1: Fresh unripe banana peels (*M. acuminata)*

2.2 Preparation of plant material

After the identification, the peels of *M. acuminata* were washed, cut into smaller pieces, and spread under the sun for some minutes for them to drain water and then placed-on cellophane and allowed to dry for 14 days under room temperature. The dried peels were then pulverized (grinded) into fine powder using wooden pestle and mortar.



Fig 2: Spreading of the unripe banana peels (*M. acuminata)* under the sun



Fig 3: Dried unripe banana peels (*M. acuminata)*



Fig 4: Grinded ripe and unripe banana peels *(M. acuminata)*

2.3 Preparation of ethanolic extract (maceration and extraction)

Cold extraction method (Maceration) was employed in this study following the procedure described by Hidayat and Wulandari (2021) [47]. In this process 240g plant materials were macerated in 1000 mL of 99% concentrated ethanol in an airtight container and kept at room temperature for 72 hours (3 days). The ethanolic suspension was filtered using a filter net and filter paper and the extract was evaporated in a water bath at 40° C for 48 hours and stored in a beaker covered with aluminum foil for bioassay immediately after the evaporation was completed.

2.4 Collection of test organism

Juveniles of *Clarias gariepinus* were collected from Akwa Ibom State University fish farm, Obio Akpa Akwa Ibom State, Nigeria and were acclimatized in a re-circulatory plastic aquarium measuring 25×13×8.3 cm^3 containing hatchery water for 24hours in the Fisheries and Aquaculture laboratory of Akwa Ibom State fish farm. This acclimation period helped to stabilize the fish and minimize stress resulting from collection and transportation (George, *et. al*., 2024 a, b) [35, 36].



Fig 5: Juveniles of *Clarias gariepinus*

2.5 Preliminary test
Prior to commencement of actual experiment, a preliminary test (range finding test) was conducted using varying concentrations (0, 10, 12, 14, 16 mg/l) to determined appropriate concentrations levels for the bioassay. Each of the aquarium had a replicate to ensure reliability of results. Each of the Ten (10) plastic aquaria were filled with two liters of hatchery water and 10 *Clarias gariepinus* juveniles were stocked in each aquarium. The ethanolic extract of *Musa acuminata* was added at varying concentrations (0, 20, 22, 24, 26 mg/l) and the setup was maintained for 96 hours for mortality examination (George, *et. al*., 2013 a; b; 2014) [33, 34, 39].

2.6 Monitoring of water quality
Water Quality Parameters were monitored prior to commencement of the experiment and periodically thereafter, following the Standard Method (APHA, 2005). Parameters that were investigated included Dissolve Oxygen (DO), pH, Temperature (^{0}C) and conductivity. Temperature and pH were measured using portable pH /Ec/ TDs/ Temperature HANNA, H1 991301 Model instrument while dissolve oxygen was measured using 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 *Musa acuminata* during the study period were determined using the following formula:
% mortality =n/N × 100 (Chan, 1977) [19].
Where:
n = number of dead fish per aquarium per concentration
N = Total Individual Stocked
The difference between dead fish and survivors gave the percentage survival of the juveniles at the end of the experiment (96 hours) (George *et.al*, 2023c; d) [41, 42].

2.7.1 Determination of mortality lethal median concentration (96 hours Lc_{50})
The effects of the various concentrations of the ethanolic extract of *M. acuminata* on the juveniles of *C. gariepinus* were determined by graphical method (Probit Level Determination as recommended by Finney (1971). The Lethal Median Concentration (LC_{50}) at 96 hours was estimated from the graph, representing the concentration expected to cause mortality in 50% of the juveniles. Similarly, the concentration corresponding to 50% mortality at the end of the 96-hour test was determined at the probit level.

2.7.2 Behavioral and morphological measurement
Behavioral cum morphological responses of *C. gariepinus* juvenile exposed to *M. acuminata* were monitored and measured daily according to OECD (2014). In addition to the acute doses, the controls without toxicant exposure were observed as a guide for the assessment of any behavioural and morphological changes. The responses monitored were irregular swimming, excessive mucus secretion, increased air gulping, vertical erection, respiratory distress and change in skin colour. Each experimental tank was observed for the exposure durations.

2.7.3 Collection of samples for histopathological examinations
The gills and liver tissues were isolated from the test animal and fixed in formalin saline for 48 hours. The fixed tissue was processed manually through graded ethanol, cleared in xylene impregnated and embedded in paraffin wax. Sections of the tissue sample were cut with a rotary microtome and stained by hematoxylin and eosin technique. Prepared tissues were finally observed for pathological changes using a microscope at x100 and x400 magnification.

2.8 Data analysis
The results of the respective concentration effects of the ethanolic extract of *M. acuminata* were presented in tables. One-way analysis of variance (ANOVA) was used to test for significant difference between the varying concentrations in both batches (batch A and batch B) at the probability level of ($p < 0.05$). Probit analysis was done using SPSS version 20.0.

3. Results
3.1 Initial water quality parameters
The initial water quality parameters prior to commencement of the toxicity studies are shown in Table 1. The values of dissolved oxygen (4.2 mg/l), temperature (28.0 ^{0}C), pH (5.58) and conductivity (0.05 µS) observed 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 (^{0}C)	pH	Conductivity (µS)
Clarias gariepinus (Juveniles)	4.2 mg/l	28.0 ^{0}C	5.58	0.05 µS

3.2 Variation in physico-chemical parameters of the test media with *Clarias gariepinus* Juveniles as test organism during the experimental period (96 hours)

Variation in the water quality parameters (DO, temperature, pH, conductivity) of the test media at the different concentrations and exposure time is presented Figure 6-9.

3.2.1 Dissolved oxygen (mg/l)

The lowest concentration of dissolved oxygen was observed in the control of the unripe peels extract at 72 hours of test while the highest DO level was observed in the 0 mg/l concentration during the 24 hours of test. The variation of DO at different time intervals and concentrations is shown in Fig. 6. At 24, 48, 72 and 96 hours of test respectively, significant variation ($p < 0.05$) was observed across the various concentrations of the extract.

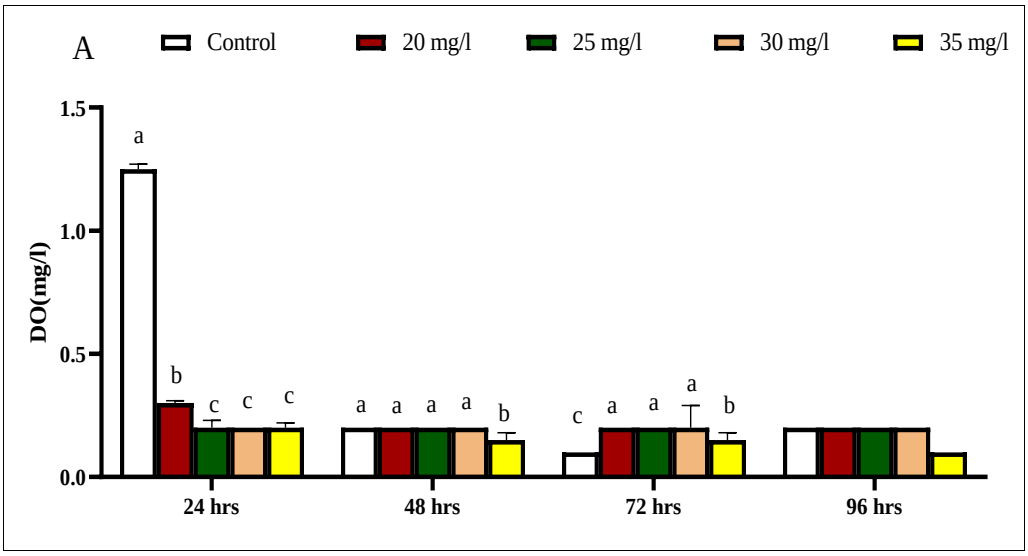


Fig 6: Dissolved oxygen variations during the 96 hours of test (unripe banana peels extract)

3.2.2 Temperature (^{o} C)

Variations in temperature in the unripe banana peel extract showed significant variation ($p < 0.05$) across all the concentrations and time interval (Fig. 7).

3.2.3 pH

The value of pH was observed to vary during the experimental period of 96 hours. Significant variations ($p < 0.05$) were observed across all the concentrations with respect to the different time intervals (Fig. 8). However, it was observed that the values of pH were general high 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 across the different time intervals. However, significant variations ($p < 0.05$) were observed in all the concentrations across the different time intervals (24, 48, 72 and 96 hours of test) (Fig. 9).

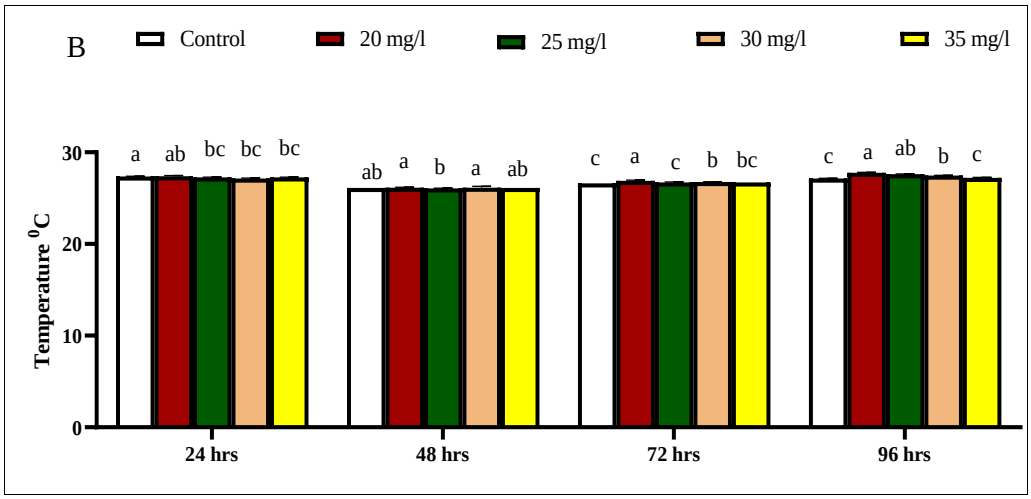


Fig 7: Temperature variations during the 96 hours of test (unripe banana peels extract)

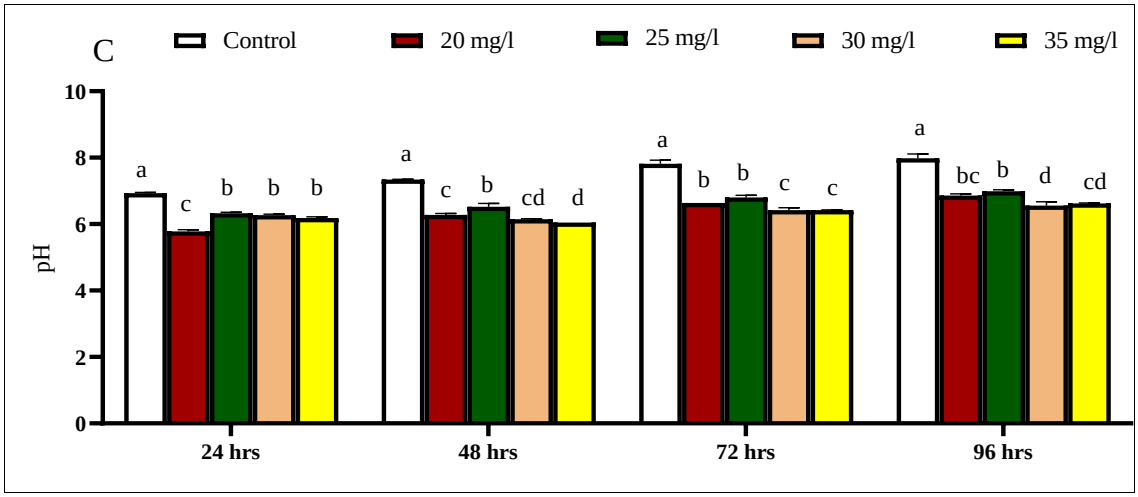


Fig 8: pH variations during the 96 hours of test (unripe banana peels extract)

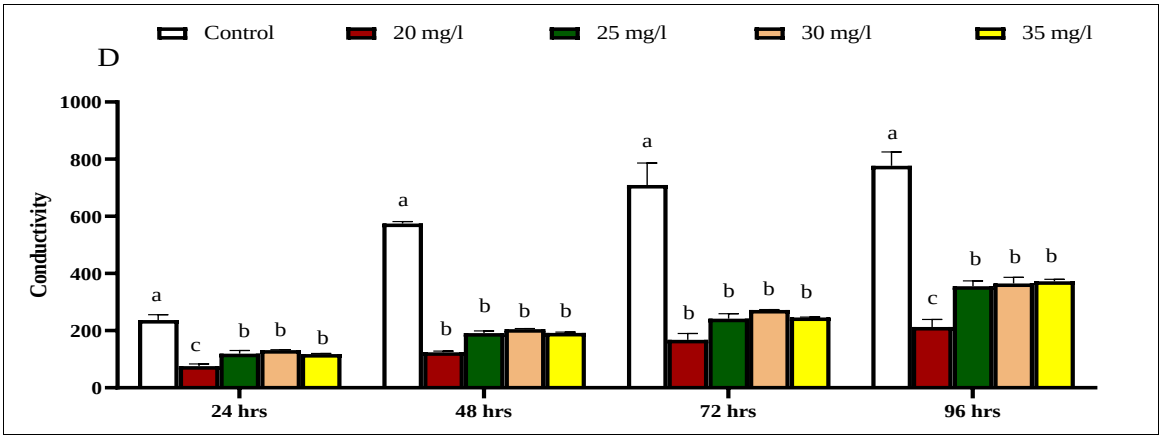


Fig 9: Conductivity variations during the 96 hours of test (unripe banana peels extract)

3.3 Percentage mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanolic extract of unripe peels of *Musa acuminata* at the end of the experiment (96 hours) (Preliminary Test).

During the preliminary test of the toxicity of the different concentration of the ethanoic extract of ripe and unripe peels of *Musa acuminata* on the test organism, no mortality was recorded in the control (0 mg/l) tank resulting in 100% survival of the test organisms in both batches A and B respectively during the 96th hour exposure periods. Similar results were observed across the various concentrations (5, 10, 15 and 20 mg/l) in both batches A and B respectively during the 96th hour exposure periods.

3.4 Summary of the percentage mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanoic extract of Ripe and Unripe peels of *Musa acuminata* at the end of the experiment (96 hrs.).

The percentage mortality and survivors of *C. gariepinus* juveniles at the end of the test period in each of the concentrations are shown in Table 2 for the two batches of the experiment. In the 0 mg/l and 20 mg/l concentration of the extract, no mortality was recorded throughout the test period in both batches A and B for unripe banana peels extract.

In the 25 mg/l concentrations of the peels extract, 20% mortality was recorded in both batches leaving behind 80% survivors in both batches respectively. A mark decrease in the number of survivors was observed in the 30 mg/l concentration of the extract of *Musa acuminata* at the end of the 96 hours bioassay. 50% mortalities were recorded in both batch A and B respectively leaving behind 50% survivors. A uniform mortality of 60% mortalities was recorded in the 35 mg/l concentration in both batches at the end of the 96 hours bioassay leaving behind 40% survivor.

Table 2: Summary of the percentage mortality and survivors of *C. gariepinus* Juveniles in the different concentrations of the ethanolic extract of unripe peels of *Musa 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	**0**	0	10	100
25 mg/l	2	20	8	80	**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 96 hours LC_{50} determination for unripe peels of *Musa acuminata*

The 96 hours LC_{50} for *C. gariepinus* juveniles exposed to the different concentrations of the ethanolic extract of unripe *Musa acuminata* was determine using probit analysis. The concentrations were first transformed into log for the probit analysis (Table 3). The 96 hours LC_{50} for unripe *Musa acuminata* peels extract is given at 30 mg/l representing a log transformed concentration of 1.47 mg/l, a point where 50% of the test organisms would be killed at the end of the experiment.

Table 3: LC_{50} determination for *C. gariepinus* Juveniles at the end of the 96-hours bioassay for unripe banana peels *Musa acuminate*

Concentration (mg/l)	Log Transformation	Mortality (M)	% Mortality	Survivors (S)	% Survivor
0 mg/l	0	0	0	10	100
20 mg/l	1.30	0	0	10	100
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 peels of unripe *Musa acuminata* for 96 hours bioassay.

Behavioural response of *C. gariepinus* juveniles exposed to concentrations of ethanolic extract of unripe *Musa acuminata* peels between 24 and 96-hours exposure durations were analyzed via a semi-quantitative measurement (Table 4). After 24 h exposure, the treated groups exposed to 20-35 mg/l concentrations of the peels extract displayed slight to moderate behavioural changes such as air gulping, erratic swimming, respiratory distress, and vertical erection, while the control (0 mg/l) concentration exhibited none of these behavioural changes. Further exposure (48-96 hours.) to the peels extract promoted moderate to severe earlier stated behavioural abnormalities in the treated groups, in a concentration-dependent pattern, except for the 20 and 25 mg/l.

Table 4: Effect behavioral responses of *Clarias gariepins* Juveniles to the toxic effects of ethanolic extract of peels of unripe *Musa acuminata* during 96th 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 the different concentrations of the ethanolic extract of unripe peels of *Musa acuminata* at the end of the experiment (96 hours).

3.7.1 Gills histopathology

Gill architecture remained normal across most treatment groups. Control fish (0 mg/L) and those exposed to 20 mg/L displayed intact primary and secondary lamellae with preserved epithelial lining and vascular structures, and no pathological lesions. Fish exposed to 35 mg/L e highest concentration tested showed histological features indistinguishable from controls, with no evidence of epithelial degeneration, lamellar fusion, or hyperplasia.

Mild, focal alterations appeared only at intermediate concentrations. At 25 mg/L, a localized area of epithelial vacuolation was present within otherwise intact branchial tissue. At 30 mg/L, focal epithelial degeneration was observed within the primary filament, though surrounding lamellae retained normal morphology. These changes did not follow a concentration-dependent pattern; the absence of branchial lesions at 35 mg/L despite their presence at 25 and 30 mg/L indicates that the extract did not produce progressive gill damage across the tested range.

3.7.2 Liver histopathology

Control fish (0 mg/L) displayed diffuse hepatic steatosis with widespread hepatocellular lipid vacuolation and peripheral nuclear displacement, but no inflammatory infiltrates or

necrosis. At 20 mg/L, hepatic architecture was largely preserved with distinguishable hepatocyte cords, though dilated, congested blood vessels and mild parenchymal and sinusoidal inflammatory infiltrates were present. The 35 mg/L group showed a similar vascular pattern dilated, congested vessels but with intact parenchymal organization, minimal cellular degeneration, and no vacuolation or necrosis.

The most pronounced hepatic damage occurred at intermediate concentrations. At 25 mg/L, hepatocellular cord organization was disrupted, with degenerative parenchymal changes and loss of normal cellular integrity. The 30 mg/L group showed comparable alterations: parenchymal disorganization, hepatocellular degeneration, and disrupted cord alignment, without necrosis or haemorrhage. This pattern moderate structural damage at 25-30 mg/L flanked by milder changes at 20 and 35 mg/L mirrors the non-monotonic response observed in the gill findings and does not support a simple linear dose-toxicity relationship.

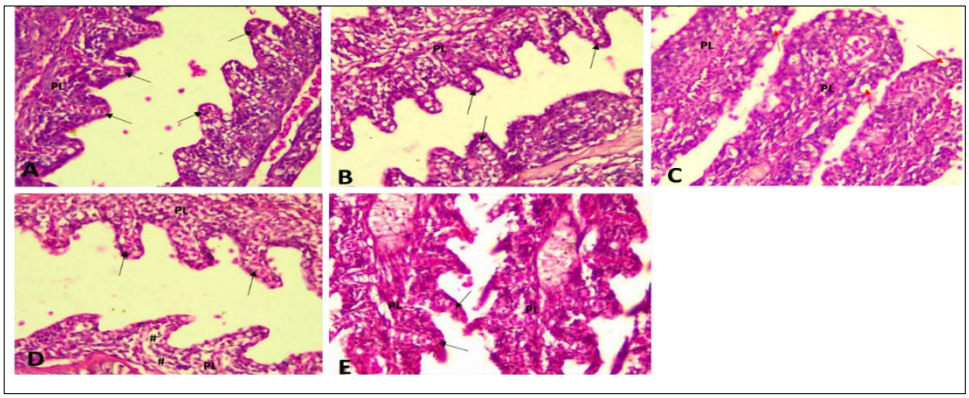


Fig 10: Representative photomicrographs of gill tissues from juvenile *Clarias gariepinus* exposed to varying concentrations of *Musa paradisiaca* peel ethanolic extract. (A) Control and (B) 20 mg/L group: normal primary lamellae with orderly secondary lamellae, intact epithelium, and preserved vascular structures; no pathological changes are evident. (C, D) Normal primary lamellae with intact epithelium and preserved vascular architecture, displaying focal epithelial vacuolation (red arrow). (E) Histological features consistent with those observed in the control and 20 mg/L groups. (H&E stain, 400× magnification)

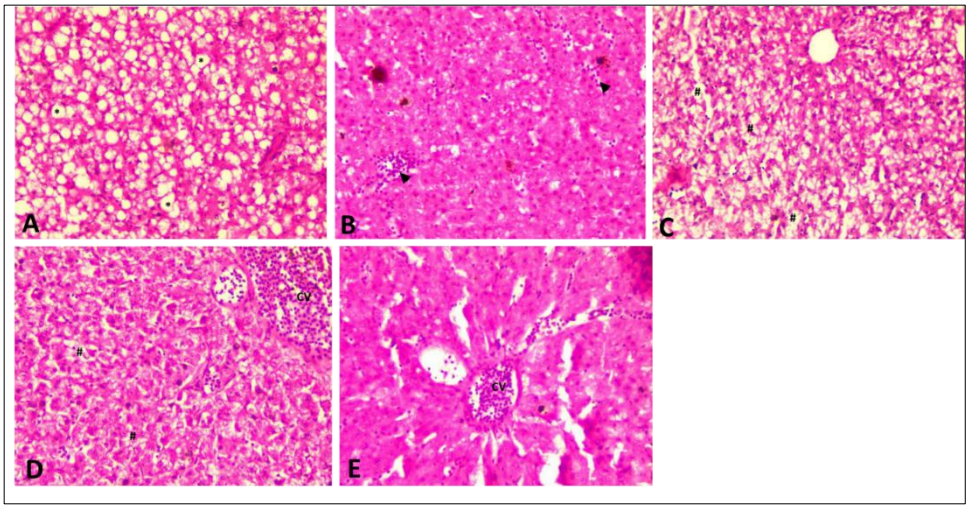


Fig 11: Representative photomicrographs of liver tissues from juvenile *Clarias gariepinus* exposed to varying concentrations of *Musa paradisiaca* peel ethanolic extract. (A) Control: diffuse steatosis (asterisk) with widespread hepatocellular lipid vacuolation and peripheral displacement of nuclei. (B, E) Dilated congested blood vessels within a relatively preserved hepatic architecture. (C, D) Distorted hepatic architecture with parenchymal degenerative changes (#), characterized by disorganization of hepatocellular cords and loss of normal cellular integrity. (H&E stain, 400× magnification)

4. Discussion

The exposure of *Clarias gariepinus* juveniles to varying concentrations of ethanolic extract of unripe peels of *Musa acuminata* has revealed significant insights into the toxicological impacts on fish physiology, particularly concerning gill and liver histology.

The gills of fish are highly sensitive organs that play important roles in respiration, osmoregulation, ion exchange, and excretion. Due to their direct contact with the aquatic environment and large surface area, they are among the first organs to respond to toxic substances in water (George *et. al.*, 2025c; d) [28, 43]. Histopathological assessment of gills is therefore widely used as a reliable biomarker for evaluating the toxic effects of pollutants and plant-derived substances in aquatic organisms (Hassan, *et al*., 2023; George *et. al*., 2024c; d; Essien-Ibok *et. al*., 2024a; b) [24, 25, 28, 31, 46].

In the present study, the control fish exhibited normal gill architecture characterized by intact primary and secondary lamellae, preserved epithelial lining, and normal vascular structures. This observation confirms that the fish used in the experiment were healthy and maintained under suitable environmental conditions. The present findings align favourably with earlier studies reported by several other authors (Sula *et al*., 2020; George *et. al*., 2015a; b) [37, 38, 65].

The gill findings reinforce the overall low-toxicity profile of the ethanolic extract. Focal epithelial vacuolation at 25 mg/L

and focal epithelial degeneration at 30 mg/L represent mild, localized cellular stress responses that contrast sharply with the severe branchial lesions' lamellar fusion, epithelial lifting, haemorrhage, and necrosis characteristic of acutely toxic plant extracts tested in the same species (Hassan *et al*., 2023) [46].

Mallatt (1985) [54], in a comprehensive statistical review of toxicant-induced gill lesions across fish species, concluded that many branchial alterations represent stereotyped physiological defense responses rather than irreversible pathology, and that cytoplasmic vacuolization ranks among the mildest ultrastructural disruptions recorded. The absence of any branchial lesion at 35 mg/L the highest concentration mirrors the hepatic pattern and further argues against progressive concentration-dependent organ damage. That both organs exhibit their mildest histological profiles at the highest tested dose, while peak injury clusters at 25-30 mg/L, suggests a systemic threshold phenomenon rather than organ-specific vulnerability. Collectively, the histopathological evidence indicates that ethanolic extract of unripe *M. paradisiaca* peel exerts limited acute tissue toxicity in juvenile *C. gariepinus* within the 20-35 mg/L range, though the non-monotonic response pattern and the confounded hepatic baseline necessitate sub-chronic exposure trials with quantitative lesion scoring (Bernet *et al*., 1999) [16, 17] and standardized lipid-controlled diets to define a robust no-observed-adverse-effect level for aquaculture application.

The hepatic findings reveal a concentration-dependent pattern that peaks at intermediate doses and attenuates at the highest exposure. Moderate hepatocellular degeneration and parenchymal disorganization at 25-30 mg/L, flanked by milder vascular and inflammatory changes at 20 mg/L and preserved architecture at 35 mg/L, constitutes a non-monotonic dose-response consistent with hormetic or adaptive phenomena documented across multiple biological systems (Calabrese and Baldwin, 2002; Agathokleous, 2018) [6, 18]. One plausible explanation is that higher extract concentrations activate hepatic detoxification pathways particularly phase I and phase II biotransformation enzymes that mitigate cellular injury more effectively than the partial response triggered at intermediate doses (Kong *et al*., 2025) [52]. The mild sinusoidal and parenchymal inflammatory infiltrates at 20 mg/L, without accompanying structural disorganization, suggest an early innate immune response to extract constituents rather than overt hepatotoxicity, a pattern reported in *C. gariepinus* exposed to sub-lethal concentrations of *Vernonia amygdalina* (Audu *et al*., 2017) [12] and *Melaleuca cajuputi* (Hassan *et al*., 2023) [46]. Importantly, diffuse steatosis in control fish identical to the baseline pathology observed in the companion ripe-peel study confirms that the lipid accumulation reflects dietary or husbandry factors rather than extract-induced injury. Verreth *et al*. (1994) [67] demonstrated that *C. gariepinus* larvae accumulate hepatic lipid exceeding 40% of hepatocyte volume under standard feeding regimens, establishing that commercial diet composition alone can produce steatotic baselines indistinguishable from low-grade toxicant effects. This shared confound across both studies underscores that hepatic steatosis in the present experimental system cannot be attributed to treatment without lipid-controlled dietary baselines.

The results strongly suggest a hormetic effect, where low to moderate doses induce cellular stress and damage, but higher doses stimulate, adaptive repair pathways, enhanced detoxification, and improved cellular stability. Similar hormetic hepatoprotection is documented in fish exposed to extracts of *Moringa*, (Adesina, and Oguntuga, 2009) [5] *Azadirachta indica* (Geetha *et. al*., 2019) [27], *Telfairia occidentalis* (Abubakar *et. al*., 2022) [2], ginger (Talpur *et al*., 2013) [66], and other plant bioactives. (Audu *et al*., 2021; Ekpeyong *et al*., 2022) [13, 21]. Thus, 35 mg/L may have activated protective pathways, reducing visible histopathological damage as observed in the present study.

The mortality response observed in juveniles of *Clarias gariepinus* exposed to varying concentrations of ethanolic extract of unripe banana peels demonstrated a clear concentration-dependent toxic effect of the extract on the experimental fish. The results showed that mortality increased progressively with increase in concentration of the toxicant and duration of exposure, indicating that the ethanolic extract possessed bioactive compounds capable of inducing toxic responses in the fish. These findings agree favorably with earlier assertion reported by several other authors (Hassan *et al*., 2023; Abubakar *et. al*., 2023; George *et al*., 2024a; b; 2025a; b) [1, 29, 30, 35, 36, 46]

At the control concentration (0 mg/L), no mortality was recorded throughout the 96-hour exposure period in both batches A and B, leaving behind 100% survival. This result confirms that the experimental conditions such as water quality parameters, handling procedures, and acclimatization techniques were suitable for the survival of the fish. The absence of mortality in the control further validates that the observed mortalities in the exposed groups were solely due to the toxic effects of the ethanolic extract of banana peels rather than environmental stress or experimental error. Similar results were observed and reported in a related study according to George *et al*., (2023a; d) [24, 32, 41].

Similarly, fish exposed to 20 mg/L concentration of the extract recorded no mortality in both batches throughout the duration of exposure. This suggests that the concentration was below the tolerance threshold of the juveniles and therefore insufficient to induce lethal effects within the test period. Although mortality was absent at this concentration, behavioural abnormalities were still observed, indicating that sublethal physiological stress had already commenced even before mortality occurred. This observation agrees with the findings of several toxicological studies (Abubakar *et. al*., 2022) [2] which reported that behavioral responses often precede mortality and may serve as early indicators of stress in fish exposed to pollutants.

At 25 mg/L concentration, 20% mortality was recorded in both batches, leaving behind 80% survivors. The mortality observed at this concentration indicates that the toxicant had begun to exert harmful physiological effects on the fish. The increase in mortality from 0% at 20 mg/L to 20% at 25 mg/L suggests that the fish tolerance limit was gradually being exceeded. The toxic action of the extract may have resulted from phytochemical constituents present in banana peels such as tannins, alkaloids, flavonoids, saponins, and phenolic compounds which are known to interfere with normal metabolic and respiratory activities in aquatic organisms. These compounds may have impaired oxygen uptake, disrupt osmoregulation, or cause tissue damage leading to gradual mortality.

The 30 mg/L concentration produced 50% mortality in the exposed fish, leaving only 50% survivors. This concentration represented the median Lethal Concentration (LC_{50}) of the toxicant after 96 hours of exposure. The LC50 value is an important toxicological index used in evaluating the potency and toxicity level of a substance. The determination of the LC_{50} at 30 mg/L with a log transformed concentration of 1.47

indicates that the ethanolic extract of banana peels possesses moderate toxicity to juveniles of *Clarias gariepinus*. At this concentration, the toxic effect of the extract was sufficiently severe to kill half of the exposed population, suggesting serious physiological disturbances within the fish. Such disturbances may include gill damage, respiratory impairment, excessive mucus secretion, nervous system dysfunction, oxidative stress, and disruption of enzymatic activities.

At the highest concentration of 35 mg/L, 60% mortality was recorded in both batches leaving behind 40% survivor at the end of the exposure period. The mortality observed at this concentration may have resulted from severe respiratory distress, loss of equilibrium, impaired metabolic functions, and eventual organ failure induced by the toxic constituents of the extract. The findings therefore confirm that toxicity of banana peel extract increases with concentration and exposure duration.

The concentration-dependent mortality pattern observed in this study is consistent with the general principle of aquatic toxicology which states that increasing toxicant concentration increases the severity of adverse biological effects. Similar trends have been reported in studies involving exposure of fish to plant extracts (Adebisi *et. al*., 2024; Adesanya *et. al*., 2024) [3, 4], industrial effluents (Isyaku and Solomon, 2016; Ivon *et. al*., 2017) [49, 50], pesticides (Momta *et. al*., 2024; Ezekiel *et. al*., 2025; Bala *et al*., 2023) [15, 26, 56], and heavy metals (Akaahan *et. al*., 2016) [8] where mortality increased progressively with increase in toxicant concentration.

The determination of the LC50 value at 30 mg/L provides valuable information regarding the toxic strength of the ethanolic extract of banana peels to *Clarias gariepinus*. The LC50 represents the concentration of a toxicant capable of killing 50% of the exposed test organisms within a specified time, in this case 96 hours. The LC50 obtained in this study suggests that the banana peel extract possesses appreciable toxicity despite banana peels being considered agricultural waste. The toxicity may be attributed to the presence of bioactive phytochemicals and organic compounds capable of exerting harmful effects on fish physiology. Ethanol extraction may also have enhanced the release and concentration of these compounds, thereby increasing their toxicity.

The log transformed concentration of 1.47 further confirms the reliability of the toxicity evaluation and provides a standardized approach for comparing toxicity with other toxicants. The LC50 value obtained in this study may therefore serve as a baseline reference for future toxicological assessments involving banana peel derivatives and aquatic organisms.

Behavioural abnormalities observed during the study were concentration-dependent and increased with duration of exposure. The control fish exhibited normal behavioural patterns such as active swimming and normal opercular movement indicating absence of stress under normal conditions. However, fish exposed to varying concentrations of 20, 25, 30, and 35 mg/L of the banana peel extract exhibited several behavioural aberrations which became more pronounced at higher concentrations. These abnormalities included erratic swimming, hyperactivity, loss of equilibrium, air gulping, excessive mucus secretion, discoloration, reduced response to stimuli, and lethargy.

Behavioural changes are often considered the earliest indicators of toxic stress in aquatic organisms because toxicants first affect the nervous and respiratory systems before causing mortality (Ekpeyong *et. al*., 2022) [21]. The observed behavioural abnormalities may therefore have resulted from impairment of neurological coordination, respiratory distress, and metabolic imbalance caused by the extract.

The severity of the behavioural responses at higher concentrations indicates that the toxic constituents of the banana peel extract interfered significantly with normal physiological functions of the fish. Air gulping and rapid opercular movement may specifically indicate oxygen deficiency or respiratory impairment resulting from gill irritation or damage. Likewise, erratic swimming and loss of balance may indicate neurotoxicity and muscular dysfunction.

The concentration-dependent behavioural responses observed in this study agree with previous findings (Ekpeyong *et. al*., 2022; Essien-Ibok *et al*., 2024a; b) [21, 24, 25] in aquatic toxicology where exposed fish exhibited increased stress behaviours with increasing toxicant concentration and exposure duration.

The dissolved oxygen concentrations varied significantly across the different concentrations and exposure periods. Oxygen concentration is one of the most important water quality parameters influencing fish survival, metabolism, respiration, and general physiological activities (Ali and Mishra, 2022). Interestingly, the dissolved oxygen concentration gradually reduced in the control tank at 72 hours of exposure, recording lower oxygen values compared to the exposed groups. This observation was unexpected because it is generally anticipated that toxicant exposure may reduce dissolved oxygen due to increased organic load and respiratory stress.

The reduction in dissolved oxygen in the control may be attributed to increased metabolic activities of the surviving fish, microbial activities, or decomposition of organic materials within the tank. Since no mortality occurred in the control, all fish remained alive and continuously consumed oxygen throughout the exposure period, which may have contributed to gradual oxygen depletion.

In the exposed tanks, lower fish activity and mortality at higher concentrations may have reduced overall oxygen demand, thereby resulting in relatively higher dissolved oxygen concentrations compared to the control. Reduced movement, and eventual mortality in exposed groups may have contributed to lower oxygen utilization by the fish. The significant variation in dissolved oxygen across concentrations and time intervals indicates that both exposure duration and toxicant concentration influenced oxygen dynamics in the experimental system.

Temperature variations observed across the different concentrations and time intervals were statistically significant. Temperature plays a crucial role in fish physiology because it influences metabolic rate, oxygen consumption, enzyme activities, and toxicity of chemicals (Kazmi *et al*., 2022) [51]. Although variations were observed, the temperature values were likely within tolerable limits for *Clarias gariepinus* since this species is known for its hardiness and ability to tolerate fluctuating environmental conditions. Minor temperature fluctuations may have resulted from changes in ambient environmental conditions during the experimental period.

The significance observed across concentrations and time intervals may indicate interactions between the extract and the aquatic environment which could slightly influence heat absorption or metabolic activities within the tanks.

The pH values varied significantly across concentrations and time intervals, with the control maintaining generally higher pH values throughout the 96-hour exposure period. The

relatively high pH in the control may indicate stable water conditions in the absence of toxic stress.

The lower pH values observed in exposed tanks may have resulted from the release of acidic metabolites, decomposition of extract components, or physiological stress responses of the fish (Michael *et al*., 2015) [55]. Ethanol extracts may contain organic acids and phenolic compounds capable of slightly lowering the pH of the aquatic medium. Changes in pH are important because they influence fish metabolism, ion regulation, and toxicity of pollutants. Slight reductions in pH in the exposed tanks may have increased physiological stress and contributed to the toxic effects observed in the fish.

Conductivity values were generally higher in the control tanks throughout the duration of the study, with significant differences observed across concentrations and exposure periods. This result was particularly interesting because it was expected that the exposed tanks would record higher conductivity values due to the presence of dissolved ions from the banana peel extract. The unexpected increase in conductivity in the control tanks may be attributed to accumulation of metabolic wastes, fish excretory products, or microbial decomposition processes occurring within the tanks. Since all fish survived in the control, continuous excretion and biological activities may have gradually increased ionic concentration and conductivity.

Conversely, mortality and reduced metabolic activities in the exposed groups may have limited ion release into the water, thereby resulting in lower conductivity values compared to the control. It is also possible that some compounds within the extract precipitated or interacted with dissolved substances, thereby reducing free ion availability in the exposed tanks. The significant conductivity variations observed in this study indicate that the banana peel extract influenced ionic composition and physicochemical properties of the test media, although the pattern differed from initial expectations.

The findings of this study clearly demonstrate that ethanolic extract of banana peels possesses toxic properties capable of inducing mortality, histological changes, behavioural abnormalities, and alterations in water quality parameters in juveniles of *Clarias gariepinus*. The study further emphasizes the importance of proper disposal and management of agricultural waste such as banana peels, especially when processed or concentrated extracts may find their way into aquatic environments. The observed toxic effects suggest potential ecological risks to aquatic organisms if such substances accumulate in water bodies. The results also confirm that behavioural responses and water quality variations are important indicators in aquatic toxicology studies and may provide early warning signs of environmental stress before mortality occurs.

4. Conclusion

The ethanolic extract of unripe banana peels exhibited concentration-dependent toxicity to juveniles of *Clarias gariepinus*, as evidence by increase mortality and behavioural abnormalities with increasing concentration and exposure duration. However, survival of some fish even at higher concentration tested indicates that the extract possesses relatively low acute toxicity and is not highly lethal to the species. Histopathological examination of the gills and liver revealed only mild to moderate alterations, while the overall structural integrity of both organs remained preserved without severe tissue damage. The observed behavioural and histological responses suggests that the extract elicited mild physiological stress responses in the fish. However, the absence of severe tissue degeneration and the preservation of normal organ architecture suggest that the observed lesions were not sufficiently severe to cause irreversible damage to the gills and liver. Therefore, unripe banana peel extract may be considered relatively safe for aquatic organism at lower concentrations and warrant further investigation as a potential aquaculture feed additive.

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