

Evaluation of the Toxic Potentials and Histopathological Variations in *Clarias gariepinus* Fingerlings Exposed to Ethanolic Extract of *Costus afer*

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ABSTRACT

This study investigates the toxicity of *Costus afer* on the histopathology of the gills of *Clarias gariepinus* fingerlings. Results indicate a concentration-dependent pattern of mortality, with significant reduction observed over time of exposure. Experimental groups exposed to *C. afer* exhibited pathological changes in the gills and behavioural abnormalities. Additionally, variations in water quality were observed following the introduction of the extract. The calculated LC50 of *C. afer* was determined to be 20.5 mg/l, providing crucial insights into the potential ecological impact of this plant extract on aquatic organisms.

Keywords: *Costus afer*, *Clarias gariepinus*, Toxicity, Histopathological changes, Fingerlings

INTRODUCTION

Fish and fish product demand is very high compared to the quantity supplied all over the world (Dada, et.al., 2019) [1]. Fish is a source of protein that is moderately cheap, available, easily processed and serves as a source of income compared to other livestock (Otoh et al., 2024 a, b) [2,3]. Globally, the demand for fish is at daily increased as the world population continues to expand whereas the supply of fish and fish products from the wild is drastically reduced due to environmental challenges and degradations leaving behind aquaculture as the only alternative source to achieving maximum production of fish to meet the protein intake of the growing human population (Otoh et. al 2024 c, d) [4,5].

Fish are ideal sentinels for detecting and monitoring aquatic pollutants or contaminants in aquatic environments (Bala, et. al., 2015) [6]. African cat fish (*Clarias gariepinus*) belongs to family Clariidae. It remained the most culturable species of significant in Nigeria and beyond (Otoh and Udoh, 2018) [7]. This is due to the unique characteristic of the species such as fast growth rate, good taste, generally accepted for consumption, high stocking density, high market price and high resistance to disease and ability to reproduce in captivity. (Nlewadim, et. al., 2011; Nya, et. al.,

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2017; 2023 a, b; Otoh, et. al., 2022) [8-12]. It is commonly and extensively cultured in man-made ponds and also occurs naturally in Nigerian natural freshwater systems.

Pollution leads to decrease in wild fisheries supplied (Adedeji and Okocha, 2011) [13]. Plant derivatives used for catching fishes are more friendly to the aquatic ecosystem and are proved economical compared to synthetic pesticides due to its ability to biodegrade (Das et. al., 2018) [14]. However, traditional fishing with plant substances and its derivatives has been shown to have negative consequences on aquatic biota, which roots to a significant ecological balance and does not encourage continuous fishing (Das et. al., 2018) [14]. Different plant species have been used arbitrarily to catch fish in lentic and lotic water bodies (Das et. al., 2018) [14]. Roots, barks, leaves, fruits and seeds are toxic parts of plants employed as fish poisons by artisanal fisherfolks (Ekpo, et. al., 2017) [15].

Water quality parameters are an integral part for the sustenance and survival of fish. Aquatic organisms are stressed due to alterations in water quality parameters (Michael et al., 2015; Ekpo et al., 2015; George and Atakpa, 2015 a, b) [16-19]. Standard water quality parameters are crucial for aquatic organisms' survival and continuity (Jonah et al., 2020; George et. al., 2020 a, b) [20-22]. Deterioration in the water quality due to the presence of impurities or contaminants may ultimately reduce the growth rate, reproductive behaviour, resistance to disease making the animals vulnerable to diseases, thereby resulting in massive mortality of aquatic animals (George and Effiom, 2018, Jonah and George, 2019; Jonah et al., 2019) [23-25].

Costus afer commonly known as African ginger or bush cane is a perennial herbaceous plant native to tropical regions of West Africa. It belongs to the family Costaceae and is characterized by its distinctive red cone-shaped inflorescence and lance-shaped leaves. *C. afer* typically, grows up to 2 meters in height and has thick erect stems (Okwu and Okwu, 2004; Anaga et al., 2004) [26,27].

Costus afer extracts may contain compounds that are toxic to aquatic organisms such as fish, invertebrates and algae. Exposure to these compounds can lead to reduced survival, growth and reproduction rate, as well as physiological and behavioral changes. High concentrations of *Costus afer* extract may disrupt the balance of aquatic communities by selectively impacting certain species over others. This could lead to shift in species composition and diversity, potentially affecting ecosystem functioning.

Decomposition of *Costus afer* extract in water bodies could alter water quality parameters such as pH, dissolved oxygen and nutrients concentrations. This may have cascading effects on aquatic organisms and overall ecosystem health. In areas where *Costus afer* grows abundantly and is introduced to the aquatic ecosystem, it may alter habitat structure by shading out native vegetation or changing sediment composition. This can impact the availability of food and shelter for aquatic organisms, ultimately affecting their abundance and distribution.

The impacts of *C. afer* on aquatic ecosystems highlights the importance of understanding its potential toxicity and implementing measures to mitigate its introduction and spread into natural water bodies. There is paucity of literature on the acute toxicity effects of ethanolic extracts of *C. afer* on African catfish (*C. gariepinus*) fingerlings. Understanding the effects of *Costus afer* on *C. gariepinus* fingerlings will help ensure compliance with regulatory standards and guidelines. Studying the toxicological effects of *Costus afer* on *C. gariepinus* will contribute to scientific understanding of how different substances interact with aquatic organisms. This knowledge can inform future research and conservation efforts.

MATERIAL AND METHODS

Collection of Plant Sample

Fresh leaves of *Costus afer* were collected for the study. The collection site of the plant was Obio Akpa in Oruk Anam Local Government Area, Akwa Ibom State. The date of Collection was 15th November, 2023. The plants material was transported to University of Uyo, Uyo, Akwa Ibom State for identification and authentication of the plants. This was done at the Herbarium in the Department of Botany, University of Uyo, Uyo with herbarium voucher No: (Effiong, UUH 4420 (Uyo).

Preparation of Plant Material

After the identification, the leaves were washed and sun dried. The leaves were shredded and spread on cellophane and allowed to dry for 72 hours under room temperature. The dried leaves were pulverized (grinded) into fine powder using wooden pestle and mortar.

Preparation of Ethanolic Extract (Maceration and Extraction)

Cold extraction method (Maceration) was used in this research according to Hidayat and Wulandari (2021) [28], in the extraction procedure, 1000ml of 99% Concentrated

Ethanol was used to Macerate 240g of the plant materials in an airtight container and kept in the laboratory under 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° Celsius for 48 hours and stored in a beaker covered with aluminium foil for bioassay immediately after the evaporation was complete.

Collection of Test Organism

Fingerling of *Clarias gariepinus* were collected from Akwa Ibom State University fish farm, Obio Akpa Akwa Ibom State, Nigeria located within latitude 5°17'N and 7°27'N, Longitude 7°27'E and 7°58'E. The climate of the area is tropical and is characterized by distinct wet and dry seasons. The vegetation of the study area is generally rainforest close to the mangrove belt. Human activities in the area include farming, hunting, boat building and sand mining. A total of two hundred (200) fingerling were collected and used for the study.

Acclimatization of Specimen's

The fingerlings were acclimated in a re-circulatory plastic aquarium measuring 25 × 13 × 8.3 Cm³ containing hatchery water for 24 hours in the fisheries and aquaculture laboratory of Akwa Ibom State fish farm. This enhanced the stability of the fingerlings from stress of collection and transportation (Udo et al, 2006).

Preparation of Experimental Aquaria

Ten (10) rectangular plastic aquaria measuring 25 × 13 × 8.3 Cm³ were thoroughly washed with tap water and properly rinsed with fresh water of similar salinity and allowed to drain dry for 24 hours on the laboratory bench based on Dede and Kagbo (2001).

Stocking of Specimen

Prior to commencement of actual experiment, a preliminary test or range finding test with varying concentration (0, 5, 10, 15, 20 mg/l) was conducted to give the actual variations in concentration to be used for the bioassay. Each of the aquarium had a replicate to ensure accuracy. Each of the Ten (10) plastic aquaria was filled with two liters of hatchery water and 10 *Clarias gariepinus* fingerlings was stocked in each aquarium. The ethanolic extract of *Costus afer* with varying concentrations (0, 21, 22, 23, 24 mg/l) was added to each stocked aquaria and allowed to stand for 96 hours for mortality examination.

Monitoring of Water Quality

Water Quality Parameters was monitored prior to

commencement of the experiment and also periodically according to Standard Method (APHA, 1998) [29]. Parameters that were monitored include dissolve Oxygen (DO), pH, And Temperature (OC). Temperature and pH were measured using portable pH /Ec/ TDs/ Temperature HANNA, H1 991301 Model instrument while oxygen was measured using digital portable analyser JPB - 607A from "Search Tech Instrument".

Monitoring of Specimen for Mortality

The effects of the various concentration of the ethanolic extract of *C. afer* on the fingerlings was monitored on a 24 hours' basis for 96 hours as recommended by Udo et. al., (2006) [30] and Ekanem and Ekpo (2008) [31].

Determination of Mortality and Survival Rates of Fingerlings

The percentage mortality and survival rates of the fingerlings in the different concentrations of the ethanolic extract of *C. afer* during the period of study was determine using the formula;

$$\% \text{ mortality} = n/N \times 100 \text{ (Chan, 1977) [32].}$$

Where;

n = number of dead fish per aquarium per concentration

N = Total Individual Stocked

The difference between dead fish and survivors will give the percentage survival of the fingerlings at the end of the experiment (96 hours) (Udo et. al., 2006) [30].

Determination of Mortality Lethal Median Concentration (96 hours LC50)

The effects of the various concentrations of the ethanolic extract of plant (*C. afer*) on the fingerlings of *C. gariepinus* was determined by graphical method (Probit Level Determination as recommended by Omoregie (2002) [33], Omoregie and Ufodike (2000) [34], Ekanem and Ekpo (2008) [31] and Udo et.al. (2006) [30]. At Lethal Median Concentration LC50, after 96 hours of test, the number of fingerlings that are expected to die was determined from the graph. Similarly, the concentration that will kill 50% of the stocked fingerlings at the end of the test (96 hours) was determined at the probit level (Omoregie, (2002) [33] Omoregie and Ufodike (2000) [34], Udo et. al., (2006) [30]; Ekanem and Ekpo (2008) [31].

Behavioral Assessment of the Test Organisms in the Toxicant

Behavioral morphological responses of *C. gariepinus* fingerling exposed to *C. afer* leaf extract were monitored

and measured daily according to OECD, (2014) [34]. In addition to the acute doses, the control group without toxicant exposure were also observed as a guide for the assessment of any behavioral and morphological changes in the experimental groups. The responses monitored included irregular swimming, excessive mucus secretion, increased air gulping, vertical erection and respiratory distress. Each experimental tank was observed for the concentrations and exposure durations.

Collection of Samples for Histopathological Examinations

The gill's 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, stained by hematoxylin and eosin technique, prepared tissues were

finally observed using a microscope for pathological changes at x100 and x400 magnification.

Data Analysis

The results of the respective concentration effects of the ethanolic extract of *C. afer* was 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.

RESULTS

Initial Water Quality Parameters

The initial water quality parameters prior to stocking are shown in Table 1. Dissolved oxygen had a value of 5.8 mg/l, with a value of 29.5°C for Temperature and 6.40 for pH.

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

Fish Species	Initial physico-chemical parameters prior to stocking		
	DO (mg/l)	Temp (°C)	pH
<i>Clarias gariepinus</i>	5.8	29.5	6.40

Variation in water Quality (Physico-chemical parameters) in the test media with *Clarias gariepinus* as test organism (Batches A and B) during the experimental period (96 Hours)

Table 2 shows the variation recorded in the different physico-chemical parameters for *Clarias gariepinus* (Batches A and B) in the different concentration of the extract and time.

Dissolved Oxygen (mg/l)

Dissolved oxygen concentrations ranged between 4.00 – 5.3 mg/l in the 0mg/l concentration of the extract. The highest DO value was recorded at the 24 hours of test with the least value recorded during the 96 Hour of Test.

In the 21 mg/l concentration of toxicant, dissolved oxygen ranged between 2.8 – 4.8 mg/l. the least value was recorded during the 96 hours of test while the highest value was recorded during the 24 hours of test.

In the 22 and 23 mg/l concentration of toxicant, dissolved oxygen ranged 2.4 – 4.6 mg/l and 1.4 – 4.4 mg/l respectively. The highest and least value of DO were observe at the 24 hours of test and 96 hours of test for both concentrations

during the study duration.

In the 5 mg/l concentration of toxicant, dissolved oxygen value ranged from 1.3 – 4.2 mg/l. The highest value was recorded during the 24 hour of test and the least value was observed during the 96 hours of test.

Temperature (oC)

Temperature value were observed to range between 25.1 – 27.6 oC during the 96 hours' bioassay. The least value of 25.1 oC was recorded during the 72nd and 96th hours of test in the 24 mg/l concentration of toxicant while the highest value of 27.6 oC was recorded during the 24 hours of test in the 0 mg/l concentration of toxicant (control).

pH

The value of pH was observed to range between 5.80 – 6.31 during the 96 hours' experimental bioassay. The least value of 5.80 was recorded during the 96th hours of test in the 22 mg/l concentration of toxicant while the highest value of 6.31 was recorded during the 24 hour of test in the 0 mg/l concentration of toxicant (control).

Table 2: Summary of the variations in the physico-chemical parameters in the test media during the experimental period.

Parameters	Conc. (mg/l)	BATCH A				BATCH B			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Dissolved Oxygen (mg/l) Initial: 5.8 mg/l	0	5.3	4.7	4.4	4.0	5.3	4.7	4.4	4.0
	2	4.8	4.0	3.2	2.8	4.8	4.0	3.2	2.8
	3	4.6	2.8	2.6	2.4	4.6	2.8	2.6	2.4
	4	4.4	1.6	1.5	1.4	4.4	1.6	1.5	1.4
	5	4.2	1.5	1.4	1.3	4.2	1.5	1.4	1.3
Temperature (°C) Initial: 29.5 °C	0	27.6	26.5	26.2	25.2	27.6	26.5	26.2	25.2
	2	27.4	26.4	26.3	25.5	27.4	26.4	26.3	25.5
	3	25.5	25.4	25.3	25.2	25.5	25.4	25.3	25.2
	4	25.4	25.3	25.2	25.0	25.4	25.3	25.2	25.0
	5	25.2	25.2	25.1	25.1	25.2	25.2	25.1	25.1
pH Initial: 6.40	0	6.31	6.25	6.22	6.12	6.31	6.25	6.22	6.12
	2	6.26	6.22	6.12	6.00	6.26	6.22	6.12	6.00
	3	6.21	6.20	6.00	5.80	6.21	6.20	6.00	5.80
	4	6.21	6.22	6.22	6.12	6.21	6.22	6.22	6.12
	5	6.16	6.05	6.02	6.00	6.16	6.05	6.02	6.00

Summary of the Percentage Mortality and survivors of *C. gariepinus* Fingerlings in the different concentrations of the ethanoic extract of *Costus afer* at the end of the experiment (96 hrs.).

The percentage mortality and survivors of *C. gariepinus* fingerlings at the end of the test period in each of the concentrations are shown in Table 3 for the two batches of the experiment.

In the 0 mg/l concentration of the extract, no mortality was

recorded throughout the test period in both batches A and B. In the 21 mg/l concentration of the extract, 70 % mortality was recorded leaving behind 30 % survivors in both batches.

At the end of the 96-hour bioassay 100 % mortality was observed in the 22, 23 and 24 mg/l concentration of the extract leaving behind no test organisms in the test media for both batches (Table 3). Statistical Analysis using one-way Anova (SPSS 20.0) showed that there was no significant difference ($p > 0.05$) in mortality between the two batches.

Table 3: Summary of the Percentage Mortality and survivors of *C. gariepinus* Fingerlings in the different concentrations of the ethanoic extract of *Costus afer* at the end of the experiment (96 hrs).

Conc. of extract (mg/l)	BATCH A				BATCH B			
	Mortality (M)	% M	Survivors (S)	% S	Mortality (M)	% M	Survivors (S)	% S
0	0	0	10	100	0	0	10	100
21	7	70	3	30	7	70	3	30
22	10	100	0	0	10	100	0	0
23	10	100	0	0	10	100	0	0
24	10	100	0	0	10	100	0	0

96 Hours LC50 Determination

The 96 hours LC50 for *C. gariepinus* fingerlings exposed to the different concentrations of the ethanolic extract of *Costus afer* was determine using probit analysis. The concentrations

were first transformed into log for the probit analysis (Table 4). The 96 hours LC50 is given at 20.5 mg/l representing a log transformed concentration of 1.31 mg/l a point where 50 % of the test organisms would be killed at the end of the experiment (Figure 1).

Table 4: LC50 determination for *C. gariepinus* Fingerlings at the end of the 96-hours bioassay.

Concentration (mg/l)	Log Transformation	Mortality (M)	% Mortality	Survivor (S)	% Survivor
0	0	0	0	0	100
21	1.32	10	100	3	30
22	1.34	10	100	0	0
23	1.36	10	100	0	0
24	1.38	10	100	0	0

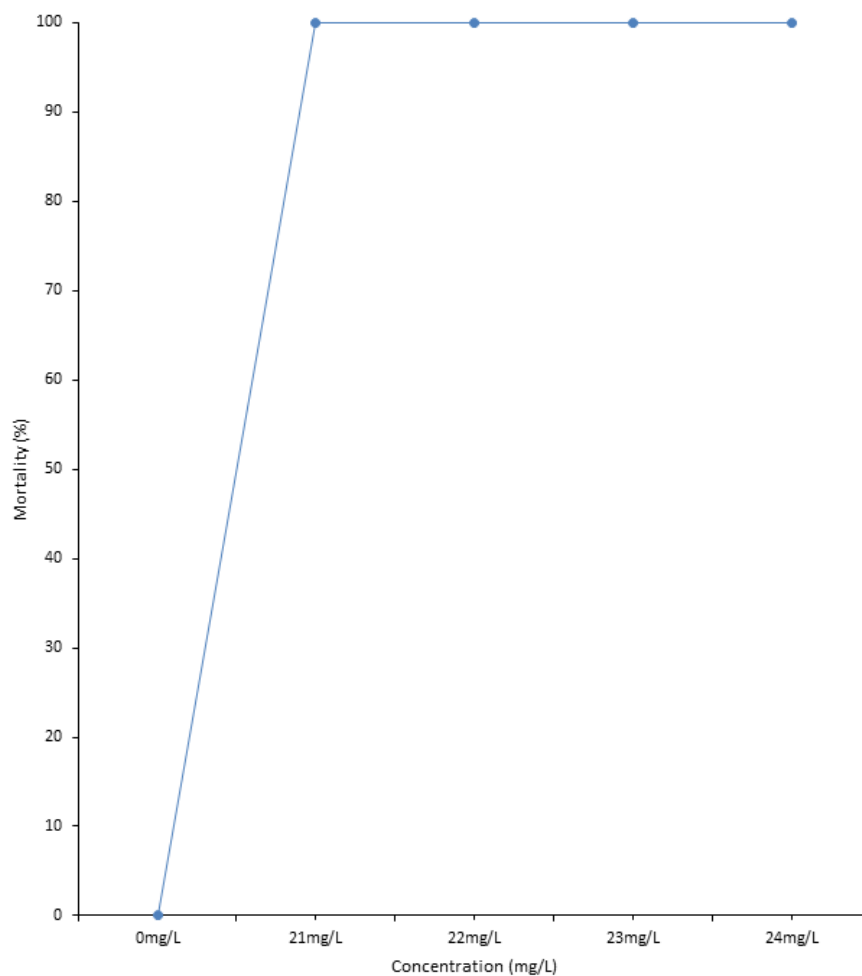


Figure 1: Probit Graph of mortality (%) against concentration.

Behavioral Responses

Behavioral response of *C. gariepinus* fingerling exposed to concentrations of ethanolic extract of *Costus afer* leaf between 24 and 96-hours exposure durations were assessed via a semi-quantitative measurement (Table 5). After 24-h exposure, the treated groups exposed to 21–24 mg/l concentrations of the plant extract displayed slight

to moderate behavioral changes such as excessive mucus secretion, air gulping, erratic swimming, respiratory distress and vertical erection, while the control and the lowest toxicant concentration exhibited none of these behavioral changes. Further exposure (48–96 hours.) to the plant leaf extract promoted moderate to severe earlier stated behavioral abnormalities in the treated groups, in a concentration-dependent pattern, except for 21 mg/l treated group.

Table 5: Effect of *Costus afer* leaf extract acute concentrations on behavior of *Clarias gariepinus* fingerlings at exposure duration intervals.

Time in hours	Conc. (mg/l)	Gulping of air	Vertical erection	Respiratory distress	Erratic swimming	Abnormal mucus secretion
24	control	-	-	-	-	-
	21	+	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	++	++	++	++	++
48	control	-	-	-	-	-
	21	+	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	++	++	++	++	++
72	control	-	-	-	-	-
	21	-	-	-	-	-
	22	+	+	+	+	+
	23	++	++	++	++	+
	24	+++	+++	+++	+++	++
96	control	-	-	-	-	-
	21	-	-	-	-	-
	22	+	+	+	+	+
	23	+++	+++	+++	+++	+++
	24	+++	+++	+++	+++	+++

Key: - None (0%); + Mild (< 10%); ++ Moderate (10 – 50 %); +++ severe (> 50 %).

Histopathology of the gill of *C. gariepinus* Exposed to the different concentrations of the ethanolic extract of *Costus afer*

The histopathological findings as shown in Figure 1 depict distinct histological changes between the control group and experimental groups exposed to different concentrations of the ethanolic extract. Gills from the control group

exhibited normal histological features, characterized by a well-organized gill arch structure. The primary lamella and secondary filament processes displayed high cellularity, and the centrally supporting cartilaginous epithelium was intact. The tissue was well-vascularized, with erythrocytes distributed throughout, indicative of normal vascular perfusion. No significant pathological changes were

observed in specimens administered with 21mg/L of the ethanolic extract. Histological analysis revealed findings consistent with normal gill architecture, comparable to the control group.

In contrast, specimens exposed to higher concentrations

of the ethanolic extract exhibited notable histological alterations. Diffuse epithelial degeneration of the gill arch, particularly affecting the lamellae, was evident across all experimental groups. Severe loss of epithelial cells was observed, suggesting extensive damage to the gill epithelium.

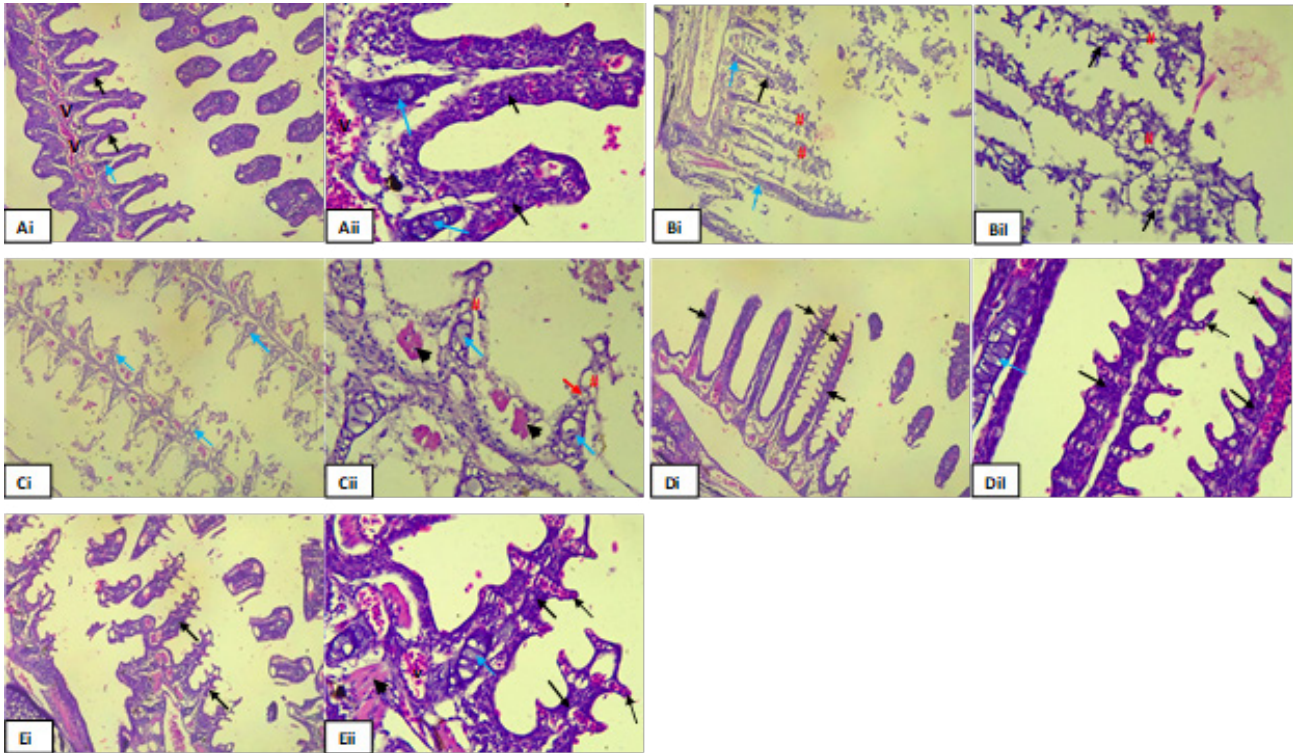


Figure 2: Photomicrograph of gills tissue section, A (i&ii) showing highly cellular Primary lamella and secondary filament at 0 mg/l of the extract, B(i&ii) gills tissue section showing highly cellular Primary lamella (**thick arrow**) and secondary filament (**thin arrow**) at 21 mg/l of the extract, C (i&ii), D (i&ii) and E (i&ii) showed degenerated gills epithelium (#) consisting predominantly the gill cartilage epithelial tissue at 22 mg/l, 23 mg/l and 24mg/l concentration of the extract respectively.

DISCUSSION

The present study investigated the toxic effect of ethanolic extract of *costus afer* leaves on the survival and histopathology of *Clarias gariepinus* fingerlings. The findings of our study confirmed the toxicity of *C. afer* leaf extract on *C. gariepinus* fingerling upon acute exposure. Prior to commencement of the experiment, basic physicochemical parameters were measured and ensured in conforms with the recommended standards for aquaculture operations. The values documented where in conformity with the approved or benchmark standards for save operations of aquaculture (Udo, 2007, Ajah 2007, George et.al., 2013a; George et.al., 2013b; George et.al., 2014a; George et.al., 2015a) [7,36-40].

The importance of this test is to ensure that it is not the presence of impurities or contaminants in the water that resulted in the toxic effects but rather the extract itself. Upon, introduction of the extract into the test media, variations in the test water were observed in the different concentrations and time of exposure. Theses variations could be attributed to the presence of bioactive compounds present in the extract, which influenced the overall toxicity observed in the experimental groups. Also, Variations in dissolved oxygen, temperature and pH levels of the test media could be attributed to the pollution potential of *C. afer* leaf extract. Presence of pollutants / toxicants in different forms can affect the living conditions and physico-chemical properties of an aquatic ecosystem (George, et. al., 2023 a) [41]. The

growth and physiological processes of aquatic organisms is primarily dependent on good water quality (George, et. al., 2015a) [40]. Variations in water quality parameters of the test media have been reported in related studies by (George et.al., 2013a; George et.al., 2013b; George et.al., 2014a; George et.al., 2015a, George, et. al., 2015 c; George et. al., 2023a, b) [37-42].

In line with standard protocols or procedure in conducting toxicological studies, it is important to carry out a range findings or preliminary test before the actual experiment. The results of preliminary findings conducted showed that no mortality was recorded in the 0 mg/l (control) concentration of the extract used for the bioassay. Similarly, 100 % survivors were recorded in the 5, 10, 15 and 20 mg/l concentration of the extract with no mortality recorded. This provided a benchmark on safe concentrations to be administered for the final toxicity test.

The treatment groups which were exposed to the different doses of the extract of *C. indica* leaves exhibited mortality rates, with the exemption of the control group. This vindicates the toxic effects of *C. indica* on the fingerlings of *C. gariepinus*. However, the toxic effects of *C. afer* were more pronounced at higher concentrations of 22, 23 and 24 mg/l concentration of the extract were no fingerlings survived at the end of the 96-h bioassay. The control group justified the toxic effects of *C. afer* as no mortality was recorded throughout the 96-h duration. Effects of contaminants or toxicants being pronounced at higher concentrations has earlier been reported by (George et.al., 2013a; George et.al., 2013b; George et.al., 2015b; George et.al., 2014b; Essien-Ibok, 2020) [37,38,43,44].

In the present study, the toxic effects observed in the fish was directly proportional to the concentration of the *C. indica* extract to which they were exposed. This implies that as the concentration of the extract increases, the adverse effects on the fish become more noticeable resulting in mortality as was observed during the study. The results of the present findings is in consonance with earlier assertions reported by previous authors; (Ayuba et. al., 2002; Calta, et.al., 2004; Adedeji et.al., 2008; Ogundiran et.al., 2010) [45-48].

The 96-h median lethal concentration value (20.5 mg/l) representing a log transformed concentration of 1.31 mg/l obtained, indicates that *C. indica* leaf extract is severely toxic to the fish model. The present study to the best of our knowledge is the first to evaluate the toxicity of ethanolic extract of *C. indica* leaf to *C. gariepinus* fingerlings. Ayuba et al. (2002) [46] stated that *Datura innoxia* leaf extract

at 120.23 mg/L caused median lethality in *C. gariepinus* fingerlings after 96 h acute exposure. *Carica papaya* seed powder at 12.9 mg/L, have been reported to produce median lethal effect in *C. gariepinus* fingerlings after 96-h exposure (Ayotunde et. al., 2010) [49]. Oribhabor and Akanse (2020) [50] reported a 96-h median lethal concentration of 0.65 mg/L *Lanchocarpus cuanescans* to *C. gariepinus*. Different plant extracts have varying levels of toxicity considering the plant parts and species, extraction methods, animal species and age (Okoro et. al., 2019; Ayuba et. al., 2012) [51,52].

Test organisms usually exhibit some behavioural changes as contaminants or toxicants is introduced into the test water. Behavioural abnormalities such as increased vertical swimming, erratic swimming, mucus secretion, respiratory distress and air gulping before death (Nwanna et. al., 2000) [53]. The pattern of behavioural changes observed in this study compared favourably with the report of (Fafioye et. al., 2004) [54] when African catfish (*Clarias gariepinus*) was exposed to *Parkia biglobosa* and *Raphia vinefera* extracts. Erratic swimming, air gulping, discoloration, loss of body equilibrium and mortality was also documented in *Clarias gariepinus* exposed to aqueous extracts of *Blighiasapida* and *Kigelia africana* (Onusiriuka and Ufodike, 1994) [55].

Histopathological examination of the gills is crucial for identifying structural alterations resulting from exposure to the ethanolic effects of *Costus afer*. The histological changes observed in the present study were concentration dependent with severe alteration been pronounced at higher concentration. Notable signs of pathological changes included disruption or loss of the gill epithelium integrity, pathological alterations in the primary and secondary gill lamellar and progressive degeneration of the gill tissues due to the exposure to the ethanolic extract of *Costus afer*. The results of this findings are similar to earlier assertion reported by George et. al., (2023, a, b, c, d) [40,56-58] when reporting on the acute toxic effects of *Phragmites capitata* on the histomorphological changes in *Clarias gariepinus* and *Oreochromis niloticus* fingerlings and *Latana camara* on the histological changes in *Clarias gariepinus* and *Oreochromis niloticus* fingerlings respectively.

The pathological changes observed in the gill of *C. gariepinus* fingerlings exposed to the ethanolic extract of *C. afer* underscore the potential toxicity of this plant extract to aquatic organisms. Understanding these pathological alterations is essential for assessing the environmental impacts of *C. afer* and implementing appropriate mitigation measures to protect aquatic ecosystem.

SUMMARY AND CONCLUSION

Following the findings of this study, we infer that ethanolic extract of the leaves of *C. afer* adversely affected the behavior of *C. gariepinus*. In addition, the plant extract induced prominent histopathological lesions in the gill tissues of the exposed fishes and subsequently mortality. The indiscriminate application of the plant extract during fishing may cause harm to both targeted and non-targeted aquatic species when in contact with the plant substance.

RECOMMENDATIONS

Further toxicological studies to elucidate the dose-response relationship between *C. afer* exposure and pathological changes in the gills of *C. gariepinus* fingerlings are encouraged. Also, expanding the histopathological examinations to assess other vital organs such as the liver and kidney to comprehensively understand the systemic effects of *C. afer* extract exposure on *C. gariepinus* and other aquatic animals.

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