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Toxicological and Haematological Effects of *Senna alata* Extract on *Clarias gariepinus* Fingerlings

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ABSTRACT The study evaluated the acute and sub-lethal effects of ethanol extract of *Senna alata* stem bark on physicochemical parameters and haematological indices of *Clarias gariepinus* fingerlings. A 96-hour acute toxicity bioassay established an LC_{50} of 11.54 mg/l (95% CI: 10.92–12.16 mg/l) and an LC_{99} of 23.30 mg/l (95% CI: 21.75–24.85 mg/l), with mortality increasing from 0% in the control to 85% at 12.6 mg/l. Sub-lethal concentrations (0.61, 0.71, and 0.81 mg/l, corresponding to 1/20th, 1/16th, and 1/14th of LC_{50}, respectively) were applied for eight weeks. Physicochemical parameters (pH, temperature, total dissolved solids, electrical conductivity, and dissolved oxygen) were monitored before and after extract application. Electrical conductivity differed significantly at 0.61 mg/l ($p = 0.0351$), while other parameters remained statistically unchanged, although dissolved oxygen declined progressively with increasing concentration. Haematological analysis revealed no significant changes ($p > 0.05$) in haemoglobin, mean corpuscular haemoglobin, mean corpuscular volume, packed cell volume, platelet, red blood cell, and white blood cell counts, except for a significant alteration in mean corpuscular haemoglobin concentration ($p = 0.0479$). These findings demonstrate that *S. alata* exhibits moderate piscicidal toxicity under acute exposure and induces mild physiological stress under sub-lethal conditions, which could have long-term implications for fish health and aquaculture productivity. The use of *S. alata* as a piscicide should therefore be approached cautiously to prevent unintended ecological consequences. Future studies should evaluate histopathological and biochemical stress responses to establish environmental safety limits for *S. alata* in aquaculture systems.

KEYWORDS: *Clarias gariepinus; Senna alata;* acute toxicity; sub-lethal exposure; haematological parameters; physicochemical parameters; piscicidal plant; aquaculture risk

1. Introduction

Aquaculture became the fastest-growing food production sector globally, contributing significantly to food security and livelihoods [1]. In Africa, *Clarias gariepinus* (African

sharptooth catfish) was among the most cultured species due to its high growth rate, hardiness, tolerance to diverse environmental conditions, and economic importance [2, 3]. However, anthropogenic activities, including the indiscriminate use of plant-based piscicides, threatened the sustainability of aquaculture and wild fish populations [4, 5].

The use of ichthyotoxic plants for fishing was an age-old practice among artisanal fishers in rural Africa, often preferred over synthetic chemicals because plant piscicides were biodegradable, eco-friendlier, and more accessible [3, 6, 7, 8]. The piscicidal activity of these plants was primarily attributed to bioactive phytochemicals such as alkaloids, tannins, saponins, and anthraquinones, which interfered with fish respiratory, circulatory, and nervous systems [9, 10]. Several plant extracts, including *Adenium obesum* [11], *Anogeissus leiocarpus* [12], and *Datura innoxia* [13], were documented to cause mortality and sub-lethal physiological disturbances in fish.

S. alata (L.) Roxb., commonly known as the candle bush or ringworm bush, was a leguminous tropical plant with well-documented pharmacological properties such as antifungal, anti-inflammatory, and antioxidant activities [10, 14, 15]. Its piscicidal potential had received limited scientific attention, especially concerning sub-lethal physiological effects on cultured fish species. The few existing studies focused primarily on acute toxicity, leaving a knowledge gap in understanding chronic or sub-lethal impacts on fish haematology, oxidative stress biomarkers, and overall health performance [16].

Haematological parameters served as reliable indicators of fish health and environmental stress because they reflected physiological responses to toxicants [17, 18]. Similarly, water quality alterations (pH, dissolved oxygen, and electrical conductivity) influenced toxicant bioavailability and fish metabolic responses [19]. Acute exposure provided immediate insights into toxicity thresholds (e.g., LC_{50} and LC_{99}), while sub-lethal studies revealed chronic stress indicators essential for aquaculture risk assessment [20, 21].

Despite its traditional use as a plant piscicide, little was known about the sub-lethal haematological and oxidative stress responses of *C. gariepinus* to *S. alata* exposure. Understanding these responses was critical for managing fish health in aquaculture systems and assessing potential ecological risks associated with the uncontrolled use of plant-based piscicides in Nigeria's inland waters. Therefore, this study investigated the acute toxicity (LC_{50} and LC_{99} and mortality pattern), physicochemical changes, and sub-lethal haematological responses of *C. gariepinus* fingerlings exposed to ethanol extract of *S. alata* stem bark.

2. Materials and Methods

2.1.Ethical approval.

The Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) approved the experimental protocols under ethical number ABUCAUC/2021/074. The approved procedures complied with the institutional guidelines for the ethical treatment of experimental animals.

2.2. Collection and maintenance of test fish.

A total of 300 healthy fingerlings of *C. gariepinus* (average weight: 2.02 g; standard length: 7.9 cm) were sourced from a commercial fish farm (BAL & KOL Fish Farm, Zaria, Nigeria). Fish were acclimatized for 14 days in two 30 l plastic aquaria under controlled laboratory

conditions (12 h light:12 h dark cycle). They were fed commercial Skretting® pellets twice daily at 3% body weight. Feeding was discontinued 24 h before the start of experiments, and water was renewed every 48 h to maintain optimal conditions.

2.3. Collection, identification, and extraction of plant material.

Fresh *S. alata* stem bark was collected from Zaria metropolis, Nigeria, and authenticated at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, where a voucher specimen [25] was deposited. The stem bark was shade-dried, pulverized, and subjected to cold maceration using 70% ethanol for 72 h. The filtrate was concentrated in a water bath at 100 °C, yielding 565.96 g of extract with an overall yield of 22.59% (w/w). Phytochemical screening, following Sofowora (1993) standard procedures, confirmed the presence of alkaloids, tannins, saponins, flavonoids, and anthraquinones.

2.4.Experimental design.

2.4.1. Acute toxicity test.

A static non-renewal 96-h acute toxicity test was conducted according to APHA [22] guidelines. Six concentrations were tested: 0.0 (control), 11.8, 12.0, 12.2, 12.4, and 12.6 mg/l. Each treatment and the control were replicated (T1 and T2) with 10 fish per aquarium (n = 120). Fish behavior and mortality were recorded hourly for the first 4 h, then daily for 96 h. Dead fish were removed immediately. LC_{50} and LC_{99} values were determined using R software.

2.4.2. Sub-lethal exposure.

Based on the 96-h LC_{50} (11.54 mg/l), three sub-lethal concentrations (0.61, 0.71, and 0.81 mg/l, equivalent to 1/20th, 1/16th, and 1/14th of LC_{50}) were prepared. These sub-lethal concentrations were selected following established ecotoxicological practice, where fractions (1/10th–1/20th LC_{50}) are commonly used to simulate environmentally relevant, non-lethal exposure levels [21, 23]. Preliminary trials confirmed that these concentrations did not cause mortality over the test duration. Fish were exposed for 8 weeks in 20 L aquaria under static-renewal conditions, with 10 fish per treatment in duplicate. Fish were randomly allocated to minimize selection bias. The control group was not exposed to the extract.

2.5. Physicochemical parameters monitoring.

Water quality parameters such as temperature (°C), pH, electrical conductivity (EC, µS/cm), total dissolved solids (TDS, mg/l), and dissolved oxygen (DO, mg/l), were measured before and after extract application using a YSI Professional Plus multiparameter probe. Measurements were taken every 24 h during the acute test and every 48 h during the sub-lethal test.

2.6.Haematological analysis.

At the end of sub-lethal exposure, blood samples were collected from three fish per replicate (n = 6 per treatment) using 2 mL heparinized syringes. Haematological parameters were analyzed following Dacie and Lewis (2001): haemoglobin (Hb, g/dL), packed cell volume (PCV, %), red blood cell count (RBC, ×10^6/mm^3), white blood cell count (WBC,

×10^3/mm^3), platelet count (PLT, ×10^3/mm^3), mean corpuscular volume (MCV, fL), mean corpuscular haemoglobin (MCH, pg), and mean corpuscular haemoglobin concentration (MCHC, g/dL).

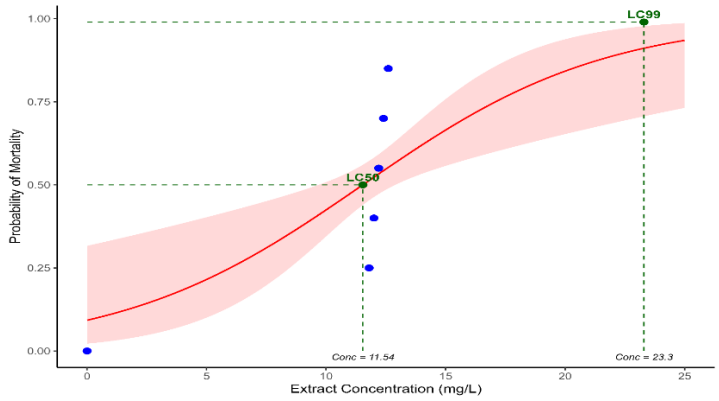
2.7. Statistical analysis.

Data were analyzed using one-way ANOVA in R. Duncan's multiple range test was selected for post-hoc analysis due to its sensitivity in detecting treatment differences with small sample sizes. Partial eta squared (η^2) was computed as an estimate of effect size to indicate the magnitude of treatment effects. Results were expressed as mean ± standard error of the mean (SEM). The 96-h LC_{50} and LC_{99} values were estimated at 11.54 mg/l (95% CI: 10.92–12.16 mg/l) and 23.30 mg/l (95% CI: 21.75–24.85 mg/l), respectively. Statistical significance was accepted at $p \leq 0.05$.

3. Results

3.1. Acute toxicity (96 h LC_{50} and mortality pattern).

The acute toxicity bioassay revealed a progressive increase in mortality of *C. gariepinus* fingerlings with increasing concentrations of *S. alata* ethanol extract (Figure 1 and Table 1). No mortality occurred in the control group, while the highest mortality (85%) was recorded at 12.6 mg/l. Regression analysis estimated the LC_{50} and LC_{99} as 11.54 mg/l and 23.3 mg/l, respectively. Behavioral abnormalities, including erratic swimming, surface gasping, and mucus secretion, were observed in approximately 70–80% of individuals at concentrations ≥12.0 mg/l, compared to 0% in the control group.



Fgure 1. LC_{50} and LC_{99} of *C. gariepinus* fingerlings exposed to *S. alata* ethanol extract (96 h).

Table 1. Mortality of *C. gariepinus* fingerlings exposed to acute concentrations of *S. alata* Extract (96 h).

Treatment	Concentration (mg/L)	Number of Fish	Total Mortality (T1)	Total Mortality (T2)	Mean Mortality (%)
Control	0.0	10	0	0	0.0
T1	11.8	10	3	2	25.0
T2	12.0	10	4	4	40.0
T3	12.2	10	5	6	55.0
T4	12.4	10	7	7	70.0
T5	12.6	10	9	8	85.0

3.2.Physicochemical parameters of water during sub-lethal exposure.

Physicochemical parameters of the test water measured before and after extract application showed no significant ($p > 0.05$) changes in pH, temperature, TDS, or DO across treatments, except for electrical conductivity (EC), which was significantly different at 0.61 mg/l [4] compared to the control. However, DO exhibited a concentration-dependent decline, particularly at 0.71 mg/l [27]. Overall values remained within the acceptable range for *C. gariepinus* culture (Figure 2). The PCA biplots of water quality parameters (Figure 2) accounted for 99.2% of variance (PC1 = 85.7%, PC2 = 13.5%). DO loaded positively on PC1, while pH, temperature, EC, and TDS had negative associations. SA0 remained near the origin, indicating stable water conditions, whereas SA0.81 shifted furthest along negative PC1, reflecting a higher organic load and possible microbial degradation of plant metabolites.

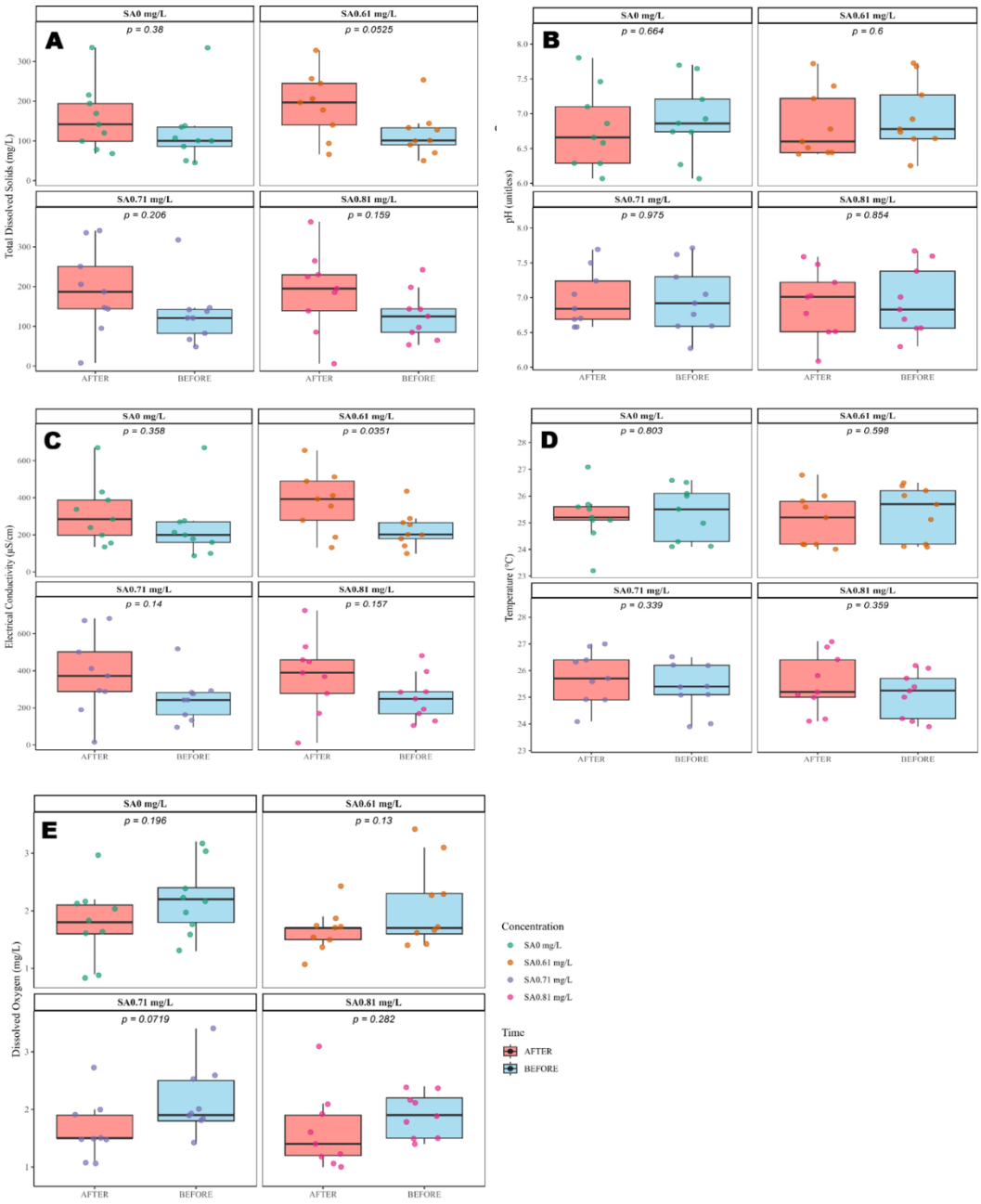


Figure 2. Physicochemical Parameters of Water before and after *S. alata* Extract Application (Sub-lethal Test): TDS (A), pH (B), EC (C), Temperature (D), DO (E).

3.3. Haematological indices of C. gariepinus after sub-lethal exposure.

Haematological responses of fish after 8 weeks of sub-lethal exposure are summarized in Figure 3. No significant alterations ($p > 0.05$) were found in Hb, MCH, MCV, PCV, PLT, RBC, or WBC across treatments compared to the control. However, MCHC was significantly altered [5], suggesting mild stress-related effects on erythrocyte indices. The PCA biplot (Figure 4) explained 98.8% of the total variance (PC1 = 82.4%, PC2 = 16.4%). WBC exhibited strong positive loading on PC1, indicating immune or inflammatory responses to the extract. In contrast, PCV and PLT had negative PC1 loadings, suggesting potential anemia and impaired clotting. RBC, Hb, MCH, MCV, and MCHC clustered closely, reflecting consistent erythrocytic responses, possibly due to hemolysis or suppressed erythropoiesis. The control group (SA0) clustered distinctly from higher concentrations, while SA0.81 was most separated along negative PC2, confirming dose-dependent haematological alterations.

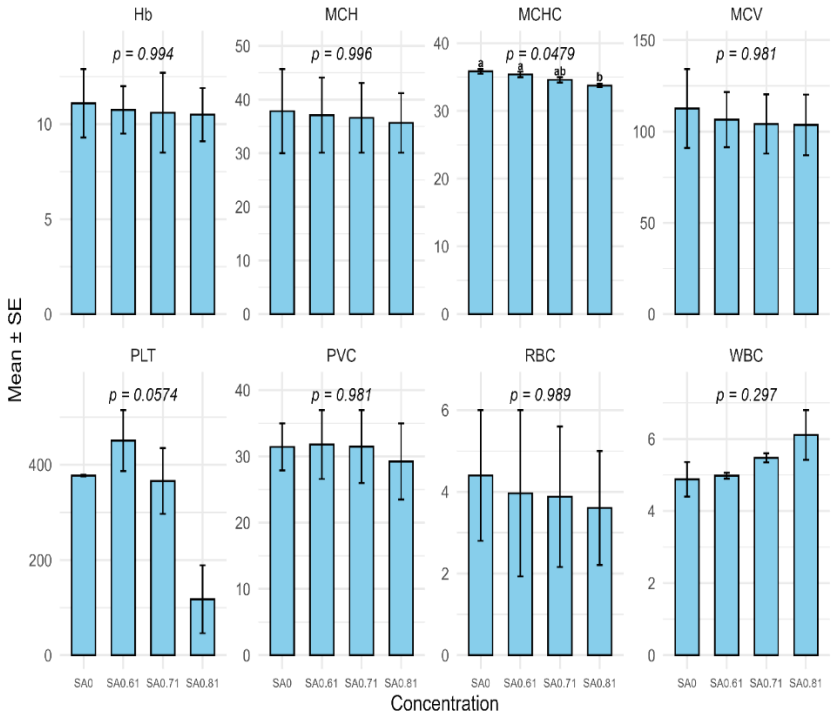


Figure 3. Haematological responses of fish after 8 weeks of sub-lethal exposure to *S. alata* stem bark.

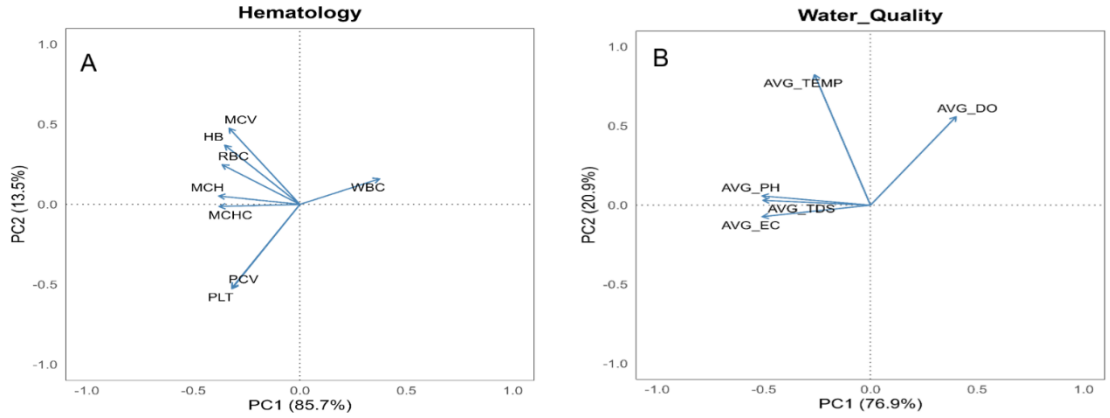


Figure 4. PCA biplots of haematological (a) and water quality (b) parameters of *C. gariepinus* exposed to *S. alata* extract.

The hierarchical clustered heatmap (Figure 5) presents the scaled interactions between water quality parameters and hematological responses of *Clarias gariepinus* exposed to varying sublethal concentrations of *Senna alata* stem bark extract. A clear functional separation was observed, with water quality variables (pH, TDS, EC, DO, and temperature) clustering distinctly from hematological indices (WBC, RBC, HB, PCV, and PLT). Concentration-dependent variations were evident, as fish exposed to lower sublethal levels (SA0 and SA0.71) exhibited relatively higher positive correlations across most parameters, while those at the highest concentration (SA0.81) showed predominantly negative responses. This pattern indicates that increasing exposure to *S. alata* extract adversely altered water quality characteristics and induced hematological stress, reflecting early manifestations of toxic effects in *C. gariepinus*.

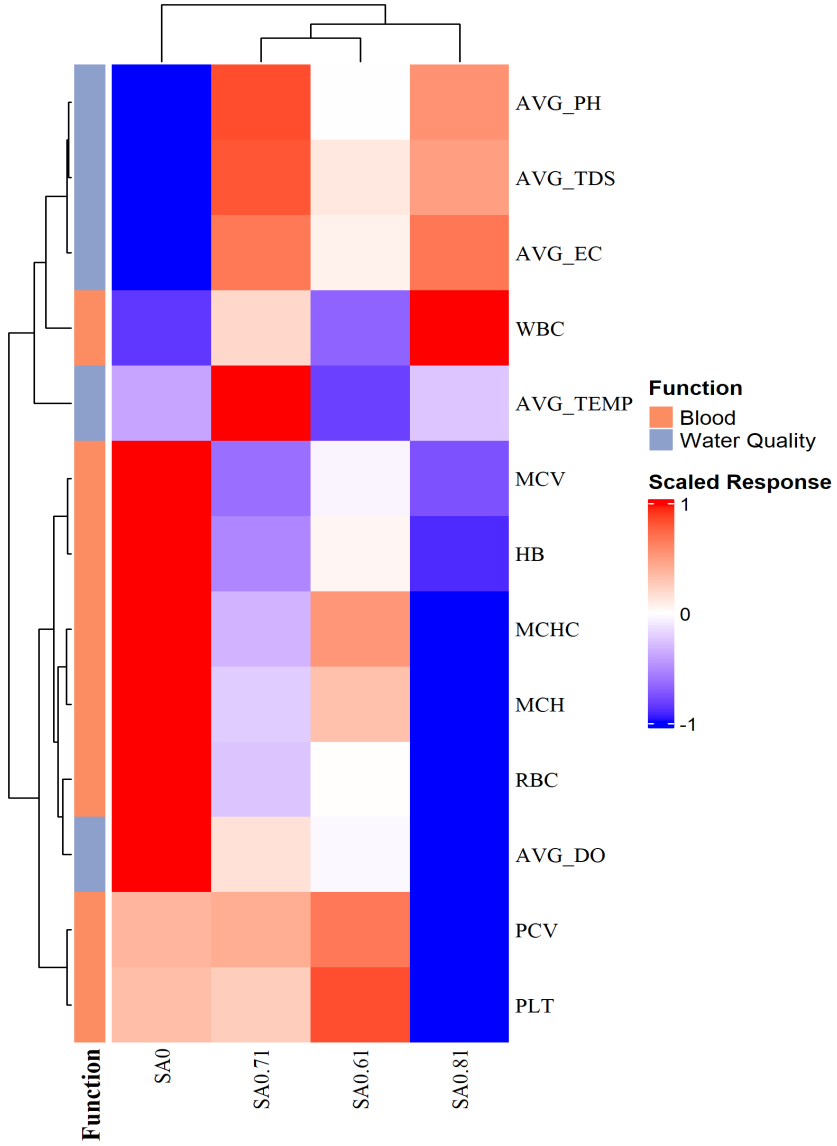


Figure 5. Heatmap showing the scaled responses of blood parameters and water quality indices in *C. gariepinus* exposed to different Sub-lethal concentrations (0, 0.61, 0.71, and 0.81 g/l) of *S. alata* stem bark extract.

4. Discussion

This study examined the acute and sub-lethal effects of *S. alata* stem bark ethanol extract on *C. gariepinus* fingerlings, focusing on oxidative stress, physicochemical changes, and

haematological responses. The findings provide valuable insights into the piscicidal potential of *S. alata* and its implications for aquaculture and fisheries management.

4.1. Acute toxicity and LC_{50}.

The 96-hour acute toxicity test revealed progressive mortality of *C. gariepinus* with increasing concentrations of *S. alata* extract, yielding an LC_{50} of 11.54 mg/l and an LC_{99} of 23.3 mg/l. This mortality pattern aligns with previous reports on the ichthyotoxic effects of plant-derived piscicides. For example, extracts of *Adenium obesum* and *Datura innoxia* demonstrated similar LC_{50} values (10–15 mg/l) in African catfish, causing mortality through respiratory distress and systemic physiological disruption [11–13]. Behavioral changes observed in this study, including erratic swimming and excessive mucus secretion, corroborate findings of [21, 24–26], attributed to gill irritation and neurotoxic effects of bioactive compounds, particularly saponins and alkaloids. The moderate LC_{50} indicates that while *S. alata* has piscicidal potential, it is less toxic than highly potent species such as *Tephrosia vogelii*, which exhibits LC50 values below 5 mg/l in teleost fish [9]. Nonetheless, the high LC_{99} reflects potential lethality at elevated concentrations, highlighting the need for cautious and regulated use by artisanal fishers to prevent unintended ecological harm.

4.2. Physicochemical alterations in sub-lethal exposure.

Physicochemical parameters measured during sub-lethal exposure showed no significant variations ($p > 0.05$) in pH, total dissolved solids (TDS), temperature, or dissolved oxygen (DO), except for electrical conductivity (EC), which differed significantly at 0.61 mg/l ($p = 0.0351$). This suggests minimal alteration of water quality at sub-lethal concentrations. However, the gradual decline in DO, particularly at 0.71 mg/l ($p = 0.0719$), may be attributed to increased microbial degradation of organic compounds in the extract [7, 27]. Optimal water quality is essential for maintaining fish health, with *C. gariepinus* thriving at DO above 4 mg/l, pH 6.5–8.5, and temperatures between 25–32 °C [19, 28]. The relatively stable physicochemical parameters suggest that chronic physiological effects of *S. alata* are more likely mediated by its bioactive compounds than by major environmental alterations. PCA and heatmap analyses further revealed concentration-dependent hematological changes, indicating that variations in packed cell volume and related indices were closely associated with exposure levels.

4.3. Responses of the haematology to sub-lethal exposure.

Haematological indices are sensitive biomarkers of fish health under toxicant stress. Most parameters (Hb, RBC, WBC, MCH, MCV, PCV, and PLT) showed no significant alterations ($p > 0.05$) after eight weeks of sub-lethal exposure, except for MCHC, which was significantly affected ($p = 0.0479$). The reduction in MCHC suggests mild anaemic or haemodilutional effects, consistent with reports from similar studies on *Clarias* species exposed to plant extracts and pesticides [16–18, 29, 30]. MCHC is a key indicator of erythrocyte oxygen-carrying capacity; significant alteration may reflect impaired erythropoiesis or increased osmotic fragility. The absence of significant changes in other haematological parameters may result from the low sub-lethal concentrations used (≤1/14th LC50), insufficient to induce severe disruption. PCA and heatmap analyses illustrated subtle but concentration-dependent

variations in blood indices, with packed cell volume changes closely aligned with exposure gradients [31–35].

4.4. Comparative insights and ecological implications.

The findings align with regional and international reports on plant-derived piscicides. In Nigeria, Audu *et al*., [12] reported mild haematological alterations in *Clarias anguillaris* exposed to sub-lethal concentrations of *Anogeissus leiocarpus*, while acute exposure caused significant mortality. Similar trends were observed globally in *Oreochromis niloticus* and *C. gariepinus* exposed to extracts of *Azadirachta indica* and *Eucalyptus camaldulensis*, which induced moderate acute toxicity but mild sub-lethal stress responses. These studies indicate that although plant-based piscicides are biodegradable, they can still cause physiological and haematological disruptions under prolonged exposure. Ecologically, the moderate toxicity of *S. alata* suggests potential risks to non-target aquatic organisms, especially under continuous or repeated application. Sub-lethal effects such as decreased erythrocyte oxygenation (low MCHC) may impair growth, reproduction, and immunity, reducing aquaculture productivity and disrupting ecosystems. In Nigeria, *S. alata* extracts are increasingly used by artisanal fishers in shallow rivers, streams, lakes, and floodplains. Prolonged exposure could suppress fish growth and survival in aquaculture ponds and alter ecological structure in natural habitats, potentially reducing yields of *C. gariepinus* and other non-target species. Thus, while *S. alata* may serve as an eco-friendly alternative to synthetic piscicides, its application should be carefully regulated and monitored.

5. Conclusion

The ethanol extract of *S. alata* stem bark possesses moderate piscicidal potential against C. gariepinus fingerlings. The 96-h acute toxicity test revealed an LC_{50} of 11.54 mg/l and LC_{99} of 23.3 mg/l, with mortality increasing proportionally with concentration. Observable behavioral stress responses, including erratic swimming, rapid opercular movement, and mucus hypersecretion, confirmed the toxic influence of bioactive compounds, primarily saponins and alkaloids. Sub-lethal exposure (0.61–0.81 mg/l for 8 weeks) resulted in stable water quality and no significant haematological alterations except for a significant change in MCHC, indicating mild erythrocytic stress. The minimal haematological disruptions suggest that *C. gariepinus* can tolerate low sub-lethal concentrations of *S. alata*, although prolonged or repeated exposure in natural systems could pose ecological risks. PCA and heatmap analyses revealed that hematological disruptions, including leukocytosis and potential anemia, were strongly linked to water quality deterioration, particularly reduced dissolved oxygen and pH fluctuations. Elevated hematological values at lower concentrations may reflect adaptive or stress responses, while the marked decline at 0.81 mg/l indicates hematological suppression and potential toxicity. These results underscore the sensitivity of blood biomarkers as indicators of aquatic toxicant exposure and highlight the need for cautious application of plant extracts in aquaculture, providing a baseline for future toxicological risk assessments. In artisanal fisheries, *S. alata* may substitute for synthetic piscicides due to its piscicidal qualities. To prevent indiscriminate application, which could endanger non-target aquatic organisms and disrupt ecological balance, its use should be strictly regulated. Sensitization of fishers on appropriate concentrations is recommended to avoid unintended ecological impacts. Long-term studies should assess histopathological, reproductive, and biochemical effects to establish safe

environmental exposure limits. Further research should compare the toxicity of *S. alata* with other plant piscicides (*Tephrosia vogelii, Azadirachta indica*) for integrated fisheries management. Regular monitoring of water quality and fish health in regions where *S. alata* is applied is also recommended to detect early ecological disturbances.

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Author Contribution

Arikpo Okoi Eteng designed and conducted the study, analyzed data, and drafted the manuscript. Jehu Auta ensured ethical compliance and supervision. Ndagi Abubakar Mohammed assisted in experimental design, Abdullateef Yusuf supervised data collection, Muhammad Onimisi Bello analyzed data, and Gabriel Ujong Ikpi suggested the plant and edited the final manuscript. All authors reviewed and approved the final version.

Competing Interest

No competing interests have been declared by the authors.

Data Availability

Data supporting this study are available from the corresponding author upon reasonable request.

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