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INDIAN ALMOND (*Terminalia catappa*) LEAF EXTRACT AS AN IMMUNOSTIMULANT IN AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

Aquaculture continues to expand globally as a major contributor to food security, yet disease outbreaks remain a critical challenge, particularly in intensive African catfish (*Clarias gariepinus*) systems. The excessive use of antibiotics in fish farms has raised concerns over antimicrobial resistance, environmental pollution, and food safety, necessitating safer, plant-based health management alternatives. This study investigated the immunostimulatory potential of *Terminalia catappa* leaf extract applied as a water additive on the haematological response and survival rate of *C. gariepinus* fingerlings. A total of 250 fish were randomly distributed into four treatments (15, 22.5, and 30 ml of extract per 30 L of culture water and a control having 0ml of extract per 30L of culture water) in a completely randomized design, with extracts prepared from shade-dried powdered leaves. Water quality parameters were monitored daily, and haematological indices were assessed using standard laboratory techniques. Results showed stable temperature and pH across treatments indicating extract safety. Dissolved oxygen increased significantly at 15 ml/30 L, while higher concentrations slightly reduced DO due to increased organic loading. Total dissolved solids varied significantly among treatments but remained within acceptable limits. Haematological analysis revealed enhanced physiological and immune responses in treated groups, with 15 ml/30 L and 30 ml/30 L producing significant increases in packed cell volume, haemoglobin concentration, red blood cell count, and white blood cell count relative to the control. Differential leukocyte counts showed higher neutrophil proportions in treated fish, suggesting stimulated innate immunity. Mortality remained negligible across all treatments with a survival rate 96.66% at 22.5ml/30L and 0ml/30L, while a survival rate of 100% was recorded at 15ml/30L and 30ml/30L of culture water confirming that the extract was safe at tested concentrations. The study demonstrates that *T. catappa* leaf extract can safely improve haematological health and modulate immune function in African catfish, supporting its potential as an plant-based immunostimulant for sustainable aquaculture.

Keywords: *Terminalia catappa*, *Clarias gariepinus*, immunostimulant, haematology, aquaculture health management

INTRODUCTION

Aquaculture has rapidly expanded in recent decades, emerging as one of the most promising sectors in addressing global food security, poverty alleviation, and income generation, especially in developing countries like Nigeria. Among the various cultivated species, *Clarias gariepinus*, commonly known as the African Catfish, has gained remarkable popularity due to its fast growth rate, adaptability to diverse environmental conditions, high feed conversion efficiency, and consumer preference (Adewolu et al., 2019). However, the intensification of catfish farming has led to increased disease outbreaks, stress, and compromised immune function, which negatively affect fish health and overall productivity (Olufemi et al., 2023). Infectious diseases caused by bacteria, viruses, and parasites have been identified as major constraints in catfish farming, leading to economic losses and reduced sustainability in the industry (Okoye et al., 2021). In Nigeria, catfish farming contributes significantly to rural livelihoods and national protein supply (Olaoye et al., 2021). The sustainability of aquaculture is increasingly threatened by disease outbreaks, particularly those caused by opportunistic pathogens in intensive culture systems (Nwosu et al., 2020).

One of the critical challenges in modern aquaculture is the overdependence on antibiotics and synthetic chemicals to combat infections. While effective in the short term, such practices have led to the emergence of antibiotic-resistant strains of pathogens, bioaccumulation in tissues, environmental degradation, and serious public health concerns (Eze et al., 2017; Usman et al., 2022). Consequently, there is an urgent need to develop environmentally safe and sustainable alternatives to improve fish health and immunity, especially under intensive farming systems.

Terminalia catappa, commonly known as Indian almond or Tropical almond, or Fruit tree, is a large tropical tree widely distributed across Asia, Africa, and the Americas. It is a multipurpose tree since almost all its parts can be utilized for human and animal benefits (Hashim et al., 2020). The dried leaves have been reported to possess therapeutic effects on infected or injured fishes (Hashim et al., 2020). In Nigeria, it is frequently found along roadsides, homesteads, and urban settlements. The plant according to Nwaeze et al. (2018) is highly regarded in ethnomedicine for its antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, and immunomodulatory properties. These bioactivities can be attributed to its rich phytochemical composition, including ellagic acid, gallic acid, flavonoids, and phenolic compounds (Chukwujekwu et al., 2015).

Indian almond leaves are not only effective when used as feed additives but also as water additives, especially in traditional Southeast Asian ornamental fish culture, where they are reputed to improve water quality and reduce stress in fish tanks. In African catfish aquaculture, this approach is being explored as a means to strengthen fish immunity and improve survival without direct medicinal intervention (Adewumi et al., 2021).

However, despite the increasing research interest, comprehensive evaluations of the efficacy, dosage, and mode of application of *T. catappa* leaves in African catfish are still limited. Previous studies have mostly focused on growth performance or basic haematological parameters, with few exploring the full immunological impact, histopathological responses, and disease resistance post-treatment. Also, variations in extract preparation methods, dosages, and application routes make it difficult to standardize recommendations for farmers (Ibrahim et al., 2020).

Thus, this study seeks to investigate the immunostimulatory potential of dried fallen Indian almond (*T. catappa*) leaves on African catfish (*Clarias gariepinus*), with a focus on the haematological parameters. The findings are expected to contribute to the development of eco-friendly, affordable, and effective health management strategies in Nigerian aquaculture, ultimately enhancing fish productivity and food security.

MATERIALS AND METHODS

Study Area

The study was conducted at the Fish Hatchery of the Department of Fisheries and Aquaculture, Federal University Dutsin-Ma, Katsina State, located in Dutsin-Ma Local Government Area] of Katsina State Nigeria. Dutsin-Ma local government lies between latitude 12°27′ 14.11′′ North and longitude 7°29′50.03′′East.

Procurement of Experimental Fish

A total of 250 healthy fingerlings of the African catfish (*Clarias gariepinus*), weighing between 1.9–3.2g, were procured from a reputable Fish hatchery in Kaduna. Fish were acclimatized for 14 days in dechlorinated water and fed a commercial diet (Skretting® starter feed, 0.8 mm pellet size, 35% crude protein, 8% lipid) at 3% body weight/day.

Preparation and infusion of *Terminalia catappa* Leaf Extract

Mature fallen leaves of *T. catappa* dried leaves were collected from the sports complex area within the University. The leaves were washed under running water to remove debris and then shade-dried at room temperature (25–28°C) for 7–10 days until a constant weight is achieved. Dried leaves were pulverized using a commercial blender (Butterfly^{R} B-592) into fine powder. A concentrated dose of 10g of powered leaves in 100ml of boiled dechlorinated water was used following the method of Hashim et al. (2020) as slightly modified. The dechlorinated water was allowed to boil after which it was dropped from the gas and immediately the weighed powdered *Terminalia catappa* leaves was then poured into the already boiled water and was continuously stirred for the first fifteen minutes, thereafter, it was allowed to cool for 3 minutes and then stirred again for the second 15 minutes, allowed to cool and stirred again for another 10 minutes, this was done continuously until 60 minutes thereafter, the solution was then filtered into clean bottles and kept for future use. A known quantity of the *T. catappa infusion* (Table 1) was then introduced into the various treatments every 48 hours.

Experimental Design

A completely randomized design (CRD) was adopted. Fish were randomly assigned into four treatment groups i.e. T1, T2, T3 and T4 respectively with T1 being 15ml of extract in 30L of culture water, T2 being 22.5ml of extract in 30L of culture water, T3 being 30ml of extract in 30L of culture water and T4 being 0ml of extract in 30L of culture water serving as the control with each treatment having 3 replicates.

Feeding and Water Management

Fish were fed twice daily in the morning between 07.00 - 08:00am and 05:00 – 06.00pm. The feeding rate was set at 5% body weight per day, and the ration was adjusted weekly based on weight gain. Water quality parameters, including temperature, dissolved oxygen, pH and TDS were monitored quantitatively every day using the Hanna instrument (Woonsocket RI USA HI 1285-5) and recorded while ammonia, ammonium, nitrate and nitrite levels were monitored qualitatively weekly using the NIFFR ammonia/ammonium/nitrate/nitrite water quality test kit. Water exchange of 50-70% was conducted every 48hours to maintain optimal water quality.

Haematological Analysis

Haematological parameters were analysed using standard techniques (Kachi, & Ugwumba, 2017): Blood samples were collected from the experimental fish into ethylene diamine tetraacetic acid (EDTA)–treated tubes for haematological analysis. Packed cell volume (PCV) was determined using the microhaematocrit method, while haemoglobin concentration (Hb) was estimated using the cyanmethemoglobin method. Red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined using an automated haematology analyzer. Differential leukocyte counts, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were determined from Giemsa-stained blood smears examined under a light microscope

Statistical Analysis

Data was analysed using SPSS version 25. One-way ANOVA was performed to determine significant differences among treatments, and means were compared using Tukey's HSD post hoc test at $p < 0.05$. the acute toxicity test (lethal concentration or LC_{50}) was determined using probit analysis.

RESULTS

The water quality parameters recorded during the culture of *Clarias gariepinus* treated with different concentrations of

dried *Terminalia catappa* leaf extract showed that temperature and pH remained statistically similar across all treatments ($p > 0.05$), Dissolved oxygen (DO) values showed significant differences, with the 15 ml/30 L treatment recording the highest mean DO (4.90 ± 2.09 mg/L) and the 30 ml/30 L and control treatments having the lowest and similar DO values (4.68 mg/L). Total dissolved solids (TDS) also differed significantly among treatments, with the 15 ml/30 L treatment showing the highest TDS (991.77 ± 40.38 mg/L) while the 22.5 ml/30 L treatment recorded the lowest value (936.26 ± 131.77 mg/L). Despite these differences, all measured water quality parameters remained within acceptable ranges for *C. gariepinus* culture.

The haematological parameters presented in Table 3 show significant variations across treatments. Packed Cell Volume (PCV) was highest in the 15 ml/30 L group (30.67 ± 0.58%) compared to the control (25.33 ± 1.15%), suggesting enhanced oxygen-carrying capacity at moderate extract levels. Haemoglobin (Hb) followed a similar trend, with significantly higher values in the 15 ml/30 L and 30 ml/30 L treatments (10.00 ± 0.00 g/dL each) compared to the control (8.60 ± 0.00 g/dL). Red Blood Cell (RBC) counts were also significantly influenced, with the 30 ml/30 L group recording the highest (5.30 ± 0.00 $\times 10^{12}$/L), while the lowest was in 22.5 ml/30 L (4.90 ± 0.00 $\times 10^{12}$/L).

White Blood Cell (WBC) counts were elevated in treated groups, particularly at 30 ml/30 L (7.77 ± 0.67 $\times 10^{9}$/L) compared to the control (6.20 ± 0.00 $\times 10^{9}$/L). Differential counts revealed treatment-specific responses, with neutrophils highest in the 30 ml/30 L group (40.00 ± 0.00%) and lymphocytes highest in the control (71.67 ± 0.58%). Monocytes and eosinophils showed no significant differences across treatments, while basophils remained absent in all groups.

Table 2: Water Quality Parameters of *Clarias gariepinus* Culture Water Treated with Different Concentrations of Dried *Terminalia catappa* Leaf Extract

Parameter	15 ml/30 L	22.5 ml/30 L	30 ml/30 L	Control (0 ml)
Temperature (°C)	28.05 ± 1.08^{a}	27.97 ± 0.92^{a}	27.94 ± 0.90^{a}	27.85 ± 0.87^{a}
DO (mg/L)	4.90 ± 2.09^{c}	4.73 ± 2.72^{b}	4.68 ± 1.93^{a}	4.68 ± 2.22^{a}
TDS (mg/L)	991.77 ± 40.38^{c}	936.26 ± 131.77^{a}	978.19 ± 132.48^{b}	951.92 ± 44.10^{b}
pH (Morning)	7.49 ± 0.31^{a}	7.52 ± 0.33^{a}	7.52 ± 0.31^{a}	7.53 ± 0.28^{a}
Survival rate(%)	100.00	96.66	100.00	96.66

Different superscript letters in the same row indicate significant differences at $p < 0.05$ (Duncan's test).

Key: DO = Dissolved Oxygen, TDS = Total Dissolved Solids

Table 3: Haematological parameters of *Clarias gariepinus* exposed to varying concentrations of *Terminalia catappa* extract.

Parameter	15 ml/30 L (T1)	22.5 ml/30 L (T2)	30 ml/30 L (T3)	0 ml/30 L (T4 control)
PCV (%)	30.67 ± 0.58^{a}	25.67 ± 0.58^{c}	29.67 ± 0.58^{b}	25.33 ± 1.15^{c}
Hb (g/dL)	10.00 ± 0.00^{a}	8.50 ± 0.10^{c}	10.00 ± 0.00^{a}	8.60 ± 0.00^{b}
WBC ($\times 10^{9}$/L)	6.77 ± 0.06^{b}	7.07 ± 0.12^{b}	7.77 ± 0.67^{a}	6.20 ± 0.00^{c}
MCH (pg)	32.33 ± 0.58^{a}	16.33 ± 1.15^{c}	19.00 ± 0.00^{b}	17.00 ± 0.00^{bc}
RBC ($\times 10^{12}$/L)	5.20 ± 0.00^{b}	4.90 ± 0.00^{c}	5.30 ± 0.00^{a}	5.17 ± 0.06^{b}
MCV (fL)	59.00 ± 0.00^{a}	53.00 ± 0.00^{c}	56.00 ± 0.00^{b}	52.00 ± 0.00^{c}
MCHC (g/dL)	32.00 ± 0.00^{b}	33.00 ± 0.00^{a}	33.00 ± 0.00^{a}	33.00 ± 0.00^{a}
Neutrophils (%)	25.67 ± 0.58^{b}	15.00 ± 0.00^{c}	40.00 ± 0.00^{a}	20.00 ± 0.00^{b}
Lymphocytes (%)	60.33 ± 0.58^{b}	70.67 ± 0.58^{a}	49.67 ± 0.58^{c}	71.67 ± 0.58^{a}
Monocytes (%)	8.67 ± 0.58^{a}	8.67 ± 0.58^{a}	8.00 ± 1.00^{a}	7.67 ± 1.53^{a}
Eosinophils (%)	4.33 ± 0.58^{a}	4.33 ± 0.58^{a}	2.33 ± 0.58^{b}	2.67 ± 1.15^{b}
Basophils (%)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.0 0.00^{a}

Keys: **PCV (%)** – Packed Cell Volume, **Hb (g/dL)** – Haemoglobin concentration, **WBC ($\times 10^{9}$/L)** – White Blood Cell count, **MCH (pg)** – Mean Corpuscular Haemoglobin, **RBC ($\times 10^{12}$/L)** – Red Blood Cell count, **MCV (fL)** – Mean Corpuscular Volume, **MCHC (g/dL)** – Mean Corpuscular Haemoglobin Concentration

DISCUSSIONS

The stable temperature and pH recorded across treatments are consistent with Boyd and Tucker (2012), who reported that herbal additives rarely disrupt carbonate buffering in aquaculture systems unless applied at high concentrations. Similarly, Nwabueze (2012) observed no significant pH shifts in

ponds treated with herbal supplements. The improved DO at 15 ml/30 L suggests that moderate *T. catappa* doses may enhance oxygen dynamics, possibly by reducing microbial oxygen demand through antimicrobial tannins. The reduced DO at higher concentrations reflects organic loading and microbial oxygen consumption, corroborating Ansa and Jiya (2002), who noted DO depletion in ponds with excess organic matter. The elevated TDS values confirm that *T. catappa* contributes dissolved organic compounds, similar to Adewolu et al. (2009), who reported increased TDS with *Moringa oleifera* extracts. While still within safe limits (<2000 mg/L), higher TDS could influence osmoregulation over prolonged exposure (Boyd 1990).

Contrary to our findings, Fafioye et al. (2017) observed slight reductions in temperature and DO when using plant extracts at higher doses, indicating that environmental context (pond vs. aquarium) may influence extract interactions. The negligible mortality recorded demonstrates safety of *T. catappa* extract at tested doses, supporting Adedeji et al. (2013), who noted that polyphenolic plant extracts confer protective effects when appropriately dosed.

The increase in PCV, Hb, and RBC in treated fish indicates improved oxygen transport efficiency and hematopoietic stimulation. This aligns with Adeyemo et al. (2022), who reported that phytogenic additives enhanced PCV and Hb in *C. gariepinus* fingerlings. Similarly, El-Sayed and Abdel-Aziz (2020) observed improved haematological indices in *Oreochromis niloticus* fed herbal supplements, suggesting a cross-species consistency in the effect of plant extracts. Our findings also agree with Akinrotimi et al. (2019), who noted elevated WBC counts in *C. gariepinus* exposed to natural immunostimulant, indicating enhanced immune responsiveness. The elevated neutrophils in this study may reflect an improved capacity to fight opportunistic infections, corroborating Gabriel et al. (2021), who linked leukocyte elevation with immune boosting from bioactive dietary compounds. Contrary to our results, Omitoyin et al. (2018) reported no significant changes in RBC counts of *C. gariepinus* fed conventional pelleted diets, highlighting that haematological improvements are extract-specific rather than diet-generic. Likewise, some contrary reports such as Musa et al. (2015) found reduced RBC and Hb in *C. gariepinus* when exposed to high doses of plant tannins, suggesting that beneficial effects may depend on dosing thresholds.

Overall, our results reinforce the potential of *T. catappa* leaf extract to enhance haematological parameters in *C. gariepinus*, improving oxygen transport and immune function. However, the variations across concentrations underscore the importance of optimal dosing for balancing erythropoietic and immunostimulatory effects.

CONCLUSION AND RECOMMENDATION

The study concludes that *Terminalia catappa* leaf extract possesses strong potential as a natural immunostimulant and water quality enhancer in aquaculture when applied at moderate levels. The extract enhanced haematological parameters, improved oxygen availability, and maintained safe water quality without causing acute toxicity. Thus, *T. catappa* is safe and beneficial at low to moderate concentrations. Overall, the study establishes that *Terminalia catappa* leaf extract has both therapeutic and environmental benefits in aquaculture systems when applied judiciously. Its ability to enhance fish haematology, maintain stable physicochemical water conditions, and improve overall physiological status highlights its potential as a sustainable alternative to synthetic additives. However, the observed variations across concentrations clearly emphasize that its beneficial effects are dose-dependent, and improper application may reverse its advantages. Therefore, optimal dosing remains critical to fully harness its value in improving aquaculture productivity and fish health.

Based on the findings of this study, the following recommendations are made:

1. Fish farmers are encouraged to adopt *Terminalia catappa* leaf extract as a natural water conditioner and immunostimulant in aquaculture systems, particularly at low to moderate concentrations, to improve fish health and water quality.
2. The application of the extract should be carefully regulated, with strict attention to dosage (such as the identified optimal level of 15 ml/30 L), to prevent oxygen depletion and excessive accumulation of organic matter in culture water.
3. Aquaculture practitioners should routinely monitor key water quality parameters such as dissolved oxygen, pH, and total dissolved solids during the use of plant-based additives to ensure that environmental conditions remain within safe limits for fish survival and growth.
4. Government and agricultural extension agencies should promote awareness and training programs on the proper use of medicinal plant extracts in aquaculture, so that farmers can apply them safely and effectively.
5. Further research should be conducted to determine the long-term effects of continuous or repeated application of *Terminalia catappa* leaf extract on fish physiology, growth performance, and pond ecosystem stability.

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