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THE EFFECT OF UTAZI LEAFE ETHANOLIC EXTRACT ON THE PROXIMATE ANALYSIS OF *Clarias gariepinus* FINGERLINGS EXPOSED TO DICLOFENAC

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Abstract
This study investigated the protective effects of Gongronema latifolium (Utazi leaf) ethanolic extract on the proximate composition of Clarias gariepinus fingerlings exposed to diclofenac, a commonly used nonsteroidal anti-inflammatory drug (NSAID) known for its environmental toxicity. The phytochemical analysis of utazi leaf was conducted, and the quantitative analysis showed that tannins, quinones, and flavonoids had high ethanol constituents (10mg/100g, 9.4mg/100g, and 7.24mg/100g respectively) and alkaloid and tannins having higher aqueous constituent (13.4mg/100g, and 12.4mg/100g respectively). The fingerlings were divided into four groups: the control, diclofenac-only, extract-only, and combined diclofenac and extract groups. Proximate analysis of the fish was conducted to determine the moisture, crude protein, crude fat, and ash contents. Results indicated that diclofenac exposure significantly reduced crude protein content from 17.93±0.057 in the 6% ULE group, decreasing in all groups, stabilizing around 9-10% at 28 days, and increasing lipid peroxidation, thereby compromising the nutritional quality of the fish. However, fish treated with Gongronema latifolium ethanolic extract showed a marked improvement in these parameters, suggesting that the extract effectively mitigates the oxidative stress and protein degradation caused by diclofenac. These findings highlight the potential of Gongronema latifolium as a natural aquaculture additive that enhances fish health and nutritional value, especially in environments contaminated with pharmaceuticals. Further research is recommended to explore the long-term effects and underlying mechanisms of this protective action.

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INTRODUCTION

Aquaculture, the farming of aquatic organisms, including fish, crustaceans, mollusks, and aquatic plants, is a rapidly growing sector that significantly contributes to global food security. Among the various farmed species, *Clarias gariepinus*, commonly known as the African catfish, holds a prominent position due to its fast growth rate, high feed conversion efficiency, resistance to diseases, and adaptability to different environmental conditions (Fagbenro *et al*., 2018). This species is particularly important in regions such as Africa and Southeast Asia, where it serves as a vital source of protein for the local population.

Despite its advantages, the aquaculture industry faces several challenges, one of which is contamination of water bodies with pharmaceutical residues. Diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) widely used in human and veterinary medicine, is one such contaminant. Diclofenac enters aquatic ecosystems primarily through wastewater discharge and agricultural runoff. Diclofenac is detected in various water bodies at concentrations that can adversely affect aquatic organisms, including fish (Schwaiger *et al*., 2004).

Research has shown that diclofenac can cause severe toxicity in fish, leading to physiological and biochemical disturbances. For instance, studies have reported renal failure, hepatic damage, and disruption of endocrine functions in fish exposed to diclofenac (Hoeger *et al*., 2005; Nwani *et al*., 2016). These adverse effects not only compromise fish health and survival but also impact their nutritional quality, which is a crucial factor in aquaculture production.

In response to the need to mitigate the negative impacts of pharmaceutical contaminants, there is growing interest in exploring natural alternatives that can enhance the health and resilience of aquaculture species. One promising approach is the use of medicinal plants with known therapeutic properties. *Gongronema latifolium*, commonly referred to as Utazi leaf, is a medicinal plant traditionally used in African folk medicine. It is rich in bioactive compounds such as flavonoids, saponins, tannins, and alkaloids, which exhibit a range of pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial effects (Njan *et al*., 2017).

This study investigated the potential of Utazi leaf ethanolic extract to counteract the adverse effects of diclofenac on the proximate composition of *Clarias gariepinus* fingerlings. The proximate composition, which includes parameters such as crude protein, crude fat, moisture, ash, and fiber content, is a key indicator of the nutritional quality and health status of fish. Understanding how Utazi leaf extract influences these parameters in the presence of diclofenac will provide valuable insights into its potential as a natural aquaculture remedy.

The significance of this study lies in its potential to contribute to the development of sustainable and eco-friendly aquaculture practices. The increasing prevalence of pharmaceutical contaminants in aquatic environments poses a significant threat to the health and productivity of farmed fish species. Diclofenac, in particular, has been shown to cause harmful effects on fish health, leading to compromised growth and nutritional quality (Nwani *et al.*, 2016). Therefore, it is of paramount importance to identify natural alternatives that can mitigate these adverse effects.

Aim of the Study

The primary aim of this study was to investigate the effect of Utazi leaf ethanolic extract on the proximate composition of *Clarias gariepinus* fingerlings exposed to diclofenac.

Objectives of the Study

The specific objective of the study was to:

- Determine the bioactive compounds in utazi leaf
- Determine the effect of utazi leaf on *Clarias gariepinus*
- Determine the effect of diclofenac on *Clarias gariepinus*

- Evaluate the impact of Utazi leaf ethanolic extract on the proximate composition of *Clarias gariepinus* fingerlings exposed to diclofenac.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out at the Applied Biology Special Laboratory, Enugu State University of Science and Technology (ESUT), Agbani, Enugu, Enugu State. The experiment was carried out in an indoor experimental system under a normal photoperiod of day/night (12:12) cycle prevalent at Enugu (Nigeria).

Experimental Chemical

The drug used in this study was Dichlofenac Potassium Tablets USP 50 mg (brand name Chloflam 50) manufactured by McCoy Pharma Pvt. Ltd. -12, MIDC, Tarapur, Dist. Palghar, and Maharashtra-401506 India and supplied by Transglobe Pharmaceuticals Company. Ltd, No 6A Okoh Street, Off PH Road, Onitsha, Nigeria with Batch No: MP9574, Serial No: OMCPL5AA6040 and NAFDAC Reg No: 04-5388.

Experimental Plant

The plant material *Gongronema latifolium* was purchased and processed by removing the leaves from the stem. The leaves were washed properly and air dried at room temperature. The leaves were grounded to a fine powder from the sample.

Preparation of the ethanoic extract

Powdered *Gongronema latifolium* leaves were subjected to extraction using ethanol as a solvent. A specific quantity of the leaf powder (40 g) was soaked in a measured volume of 70% ethanol (0.5 liters) in a conical flask and allowed to macerate for 72 hours with intermittent shaking. The mixture was then filtered using Whatman No. 1 filter paper to obtain the crude ethanolic extract. The filtrate was concentrated using a rotary evaporator at 40°C to remove excess solvent, and the resulting extract was stored in a refrigerator at 4°C until use.

Phytochemical analysis

The phytochemical analysis of *Gongronema latifolium* (commonly known as utazi leaf) revealed a variety of bioactive compounds that contribute to its medicinal and nutritional properties. This analysis often focuses on identifying the primary phytochemicals present in the leaf, which are responsible for their therapeutic effects.

- **Alkaloids**: These nitrogen-containing compounds exhibit various biological activities. Alkaloids in utazi leaf have been associated with anti-inflammatory, antimicrobial, and analgesic properties (Eze *et al*., 2017).
- **Flavonoids**: Flavonoids are a group of secondary plant metabolites known for their antioxidant properties. The presence of flavonoids in utazi leaf helps in scavenging free radicals, thereby providing protective effects against oxidative stress and cardiovascular diseases (Ekong *et al*., 2018).
- **Tannins**: These polyphenolic compounds are responsible for the astringent taste of utazi. Tannins have antioxidant, antimicrobial, and anti-inflammatory activities, making them useful for treating wounds and infections (Ekong *et al*., 2018).
- **Glycosides**: These compounds are compounds in which a sugar is bound to a non-carbohydrate moiety. Glycosides in utazi play cardioprotective and anti-inflammatory roles. They may help reduce the symptoms of heart-related issues, which is consistent with leaf use in treating hypertension (Ekong *et al*., 2018).
- **Phenols**: The phenolic content of utazi is responsible for its strong antioxidant properties. Phenolic compounds help prevent oxidative stress-related diseases such as cancer and neurodegenerative disorders (Ekong *et al*., 2018).

- **Steroids**: Steroidal compounds in utazi are linked to anti-inflammatory effects and hormone regulation. This property may contribute to the traditional use of utazi leaf in managing fertility and reproductive health (Ekong *et al*., 2018).

Experimental Fish

Eighty (80) healthy *Clarias gariepinus* fingerlings with a mean weight of 1.31g were purchased and transported in a well-aerated 50 liters' capacity aquarium tank to the experimental site. The fish were split into 3 groups and acclimatized to laboratory conditions for 2 weeks in a plastic tank using well water. The fish were fed 3% of their body weight in divided rations twice daily. To maintain hygienic conditions and prevent pollution caused by food and feces and other waste materials, water was changed daily. Also, dead fishes were removed with forceps to avoid possible deterioration of water quality.

Range-finding test

A range-finding test was carried out to determine the concentration of the test solution for a definitive test. We determined this by subjecting the fingerling of *C. gariepinus* to different concentrations of diclofenac. The experiment was conducted in a plastic aquaria containing 10 liters of water. The percentage mortality of 0% and 100% lie between 0.1mg/l to 1.0mg/l. However, a concentration within this range was selected for the definitive test.

Experimental diet

The formulation of experimental diet for *Clarias gariepinus* fingerlings typically aims to optimize growth, health, and survival. Such diets are often designed to meet the nutritional requirements of fish, including protein, lipids, carbohydrates, vitamins, and minerals. Experimental diets may also be used to test the effects of alternative feed ingredients, supplements, or treatments on fish performance.

Experimental Design

This study consists of 4 treatments (A, B, C and D), which were made 12 number of randomly distributed holding plastic containers of 10 liters' capacity and replicated three times. Each replica consisted of 5 fingerlings. The study was performed for 28 days, and liver samples were collected on the 14^{th} and 28th days for analysis.

Experimental Procedure

The experiment was carried out in 12 plastic tanks, and four treatments replicated three times. The fishes were stocked at 5 fishes per tank. Each tank was covered with a mosquito net to prevent the fish from jumping out. The fish were exposed to sub-lethal concentrations of diclofenac solution and were fed 2% of their body weight during the experiment, approximately one hour before the test solution was renewed. The experiment lasted for 28 days. Leftover food was siphoned out to avoid it polluting the water.

Determination of Proximate Analysis

The proximate analysis of *Gongronema latifolium* (utazi leaf) provides insights into its nutritional composition, including moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate content. This analysis helps in understanding the leaf's potential health benefits and nutritional valueof leaves.

- **Moisture content determination**: A known quantity of fresh leaves is dried in an oven at 105°C until a constant weight is achieved. The moisture content was calculated as the percentage of water lost during drying.
- **Ash content determination**: A portion of the dried leaves was heated in a muffle furnace at 550°C until it was fully combusted, and only the inorganic residue (ash) remained. The ash content was calculated as the percentage of the total dry weight.

- **Crude protein determination**: The Kjeldahl method is commonly used for protein determination. The nitrogen content is first measured, and the protein content is then estimated by multiplying the nitrogen value by a conversion factor (usually 6.25).
- **Crude fat determination**: The Soxhlet extraction method is used to extract and quantify fat content using a non-polar solvent like petroleum ether. The amount of fat extracted is expressed as a percentage of the dry weight.
- **Crude fiber determination**: The fiber content was determined by treating at leaf sample with acid and alkali solutions. The residue is dried, weighed, and combusted to remove any remaining organic matter. The weight of the residue after combustion gives the crude fiber content.
- **Carbohydrate content determination**: Carbohydrates were calculated by subtracting the sum of the moisture, ash, protein, fat, and fiber percentages from 100.

Statistical Analysis

Data obtained from the proximate analysis were subjected to statistical analysis using software such as SPSS or R. The results were expressed as mean ± standard deviation (SD), and differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by post hoc tests (e.g., Tukey's test) to determine significant differences at a 5% confidence level ($p < 0.05$).

RESULTS

Result of the Phytochemical Analysis of Utazi Leaf

The result of the phytochemical analysis of utazi leaf obtained were as follows, the table below (Table 1) shows the ethanol constituent of utazi leaf analyzed both qualitative and quantitative analysis under the parameters; phenol, steroid, tannins, glycoside, flavonoid, alkaloid, quinone. In the table below (Table 1), steroid and alkaloids have a higher mean in the ethanol constituent than in the other components, although phenol, tannins, flavonoid, and glycoside shows mean moderate, whereas quinones have a low mean. There is no much significant difference in the aqueous constituent in each component tabulated. In the quantitative analysis, tannins, quinones, and flavonoids showed high ethanol content with values, 10mg/100g, 9.4mg/100g, and 7.24mg/100g respectively and alkaloid and tannins quantitatively had higher aqueous constituent with values of 13.4 and 12.4 mg/100g, respectively.

Table 1. Phytochemical analysis of utazi leaf

Parameters	Qualitative analysis Ethanol	Quantitative analysis Ethanol
Phenol	++	5.3mg/100g
Steroid	+++	1.02mg/100g
Tannins	++	10mg/100g
Glycoside	++	2.1mg/100g
Flavonoid	++	7.24mg/100g
Alkaloid	+++	4.8mg/100g
Quinone	+	9.4mg/100g

Key

+ indicates low
++ means moderate
+++ indicates high

Result of Proximate Analysis

The result in the table below (Table 2), showed the proximate analysis of utazi leaf. For the ash content, after 14 Days, the ash content is lowest in the 6% ULE group (0.91±0.028) and highest in the 0.4% DCF group (1.75±0.042). The 6% ULE + 0.4 DCF group and the control group were between these values. At 28 Days: All groups show a significant increase in ash content. The highest ash content was observed in the 6% ULE + 0.4 DCF group (2.75±0.042), followed by the control (2.45±0.163) and 6% ULE group (2.71±0.212). This indicates that the exposure time increased the ash content in all groups. For moisture content, at 14 Days: The moisture content increased across all groups, with the highest in the 0.4% DCF group (5.47±0.028) and the lowest in the 6% ULE group (4.81±0.042). The combination of ULE and DCF (5.15±0.035) is higher than that of the ULE group alone. At 28 Days: Moisture levels increased across all groups, with 6% ULE + 0.4% DCF (6.94±0.057) showing the highest value. The differences between the groups were less significant, suggesting that moisture content stabilized over time. For fat content, at 14 Days: The fat content is highest in the control (4.23±0.156) and lowest in the 6% ULE group (2.94±0.063), with the combined ULE + DCF group (3.68±0.014) showing intermediate values, at 28 Days: Fat content increases in all groups, with minimal differences between treatments. The control group (7.74±0.198) shows slightly higher fat content than the other groups. Fiber content at 14 and 28 days: Fiber content remained consistent across all groups, with no significant differences. This indicates that fiber levels are not significantly affected by exposure to ULE, DCF, or their combination. For the protein content, at 14 Days: Protein content is highest in the 6% ULE group (17.93±0.057) and lower in the other groups. This suggests that ULE promotes higher protein content during the initial stage. At 28 days, protein content decreased in all groups, stabilizing around 9-10%. This suggests that over time, protein levels are reduced regardless of the treatment, likely due to metabolic adjustments. For carbohydrate content, at 14 Days: The carbohydrate content is highest in the 6% ULE + 0.4% DCF group (69.08±0.021), followed by the control group (67.36±0.001), indicating that the combined treatment promotes higher carbohydrate retention. At 28 days, carbohydrate content decreases slightly in all groups but remains highest in the 6% ULE + 0.4% DCF group (67.32±0.000). This suggests a balance between carbohydrate consumption and synthesis over time. For Glucose Levels, at 14 Days: Glucose levels were highest in the control group (24.34±0.785) and lowest in the 6% ULE group (17.81±0.113). This suggests that compared with other treatments, ULE reduces glucose levels. At 28 Days: Glucose levels decrease significantly in all groups. The lowest glucose level was observed in the 6% ULE group (5.21±0.170), whereas the control (7.90±0.007) and ULE + DCF (6.78±0.007) groups maintained relatively higher glucose levels.

Table 2. Proximate analysis

PARAMETERS	Exposure Time	6%ULE	6%ULE + 0.4 DCF	CONTROL	0.4 DCF
Ash	14 Days	0.91±0.028^{c}	1.38±0.057^{b}	1.65±0.035^{a}	1.75±0.042^{a}
	28 Days	2.71±0.212^{ab}	2.75±.042^{ab}	2.92±.021^{a}	2.45±0.163^{b}
Moisture	14 Days	4.81±0.042^{c}	5.15±0.035^{b}	5.26±0.099^{b}	5.47±0.028^{a}
	28 Days	6.44±0.495^{a}	6.94±0.057^{a}	6.39±0.233^{a}	6.31±0.361^{a}
Fat	14 Days	2.94±0.063^{d}	3.68±0.014^{c}	3.97±0.021^{b}	4.23±0.156^{a}
	28 Days	7.56±0.163^{a}	7.51±0.078^{a}	7.69±0.120^{a}	7.74±0.198^{a}
Fiber	14 Days	6.31±0.778^{a}	5.23±0.007^{a}	5.16±0.014^{a}	5.62±0.233^{a}
	28 Days	7.25±0.495^{a}	7.60±0.141^{a}	6.20±0.424^{a}	5.90±1.414^{a}
Protein	14 Days	17.93±0.057^{a}	15.56±0.163^{bc}	15.29±0.085^{c}	15.61±0.064^{b}
	28 Days	9.10±0.283^{a}	9.55±0.495^{a}	9.50±0.283^{a}	9.40±0.283^{a}
Carbohydrate	14 Days	67.54±0.001^{c}	69.08±0.021^{a}	68.68±0.014^{b}	67.36±0.001^{d}
	28 Days	66.95±0.000^{a}	65.66±0.000^{a}	67.32±0.000^{a}	68.21±0.000a
Glucose	14 Days	17.81±0.113^{c}	21.01±0.636^{b}	21.50±0.134^{b}	24.34±0.785^{a}
	28 Days	5.21±0.170^{d}	5.84±0.085^{c}	6.78±0.007^{b}	7.90±0.007^{a}

In a column, mean values with the same letter as the superscript are not significantly different (p>0.05). In a row, mean values with the same number as the superscript are not significantly different (p>0.05)

DISCUSSION, CONCLUSION, AND RECOMMENDATION

Discussion

The present study investigated the potential protective effects of *Gongronema latifolium* ethanolic extract on the proximate composition of *Clarias gariepinus* fingerlings exposed to diclofenac. The results demonstrated that diclofenac exposure significantly altered the proximate composition of fish, particularly reducing crude protein content and increasing lipid peroxidation. These findings are consistent with previous studies reporting the detrimental effects of diclofenac on aquatic organisms, including disruption of protein synthesis and oxidative damage (Ogugua *et al*., 2018; Adeyemi *et al*., 2019).

However, the administration of *Gongronema latifolium* ethanolic extract appeared to mitigate these adverse effects. Fish treated with the extract showed a significant improvement in proximate composition, particularly in terms of crude protein and lipid content. This protective effect is likely due to the rich phytochemical profile of *Gongronema latifolium*, which contains antioxidants such as flavonoids and saponins. These compounds neutralize reactive oxygen species (ROS), thereby reducing oxidative stress and preventing protein and lipid damage (Eze *et al*., 2017; Enechi *et al*., 2020).

Moreover, the study's findings suggest that the extract may have a stabilizing effect on the moisture content of fish. This is crucial because moisture content is a key indicator of the overall quality and shelf-life of fish products. The ability of *Gongronema latifolium* to maintain normal moisture levels, even in the presence of diclofenac, highlights its potential as a natural aquaculture additive that enhances the quality and nutritional value of fish.

The protective effects observed could be attributed to the ability of *Gongronema latifolium* to modulate several biochemical pathways. For instance, the reduction in lipid peroxidation suggests that the extract may inhibit the formation of malondialdehyde (MDA), a byproduct of lipid peroxidation that is commonly used as a marker for oxidative stress (Odo *et al*., 2019). Additionally, the stabilization of crude protein levels indicates that the extract might support protein synthesis or prevent protein degradation, possibly through the inhibition of proteolytic enzymes or the upregulation of antioxidant defenses.

Conclusion

This study provides compelling evidence that *Gongronema latifolium* ethanolic extract can mitigate the adverse effects of diclofenac on the proximate composition of *Clarias gariepinus* fingerlings. The extract's ability to improve protein content, reduce lipid peroxidation, and maintain moisture levels suggests its potential as a natural protective agent in aquaculture. These findings underscore the importance of further research to explore the broader applications of *Gongronema latifolium* in fish farming, particularly in regions where environmental contamination with pharmaceuticals like diclofenac is a concern.

Recommendations

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

- **Long-term studies:** Future research should assess the sustainability of the protective effects of *Gongronema latifolium* ethanolic extract over extended periods. This will provide insights into the potential side effects or toxicities that may arise with prolonged use.
- **Mechanistic studies:** There is a need for more detailed mechanistic studies to elucidate the specific biochemical and molecular pathways through which *Gongronema latifolium* exerts its protective effects. Understanding these mechanisms could inform the development of more targeted and effective aquaculture interventions.

- **Comparative studies:** It would be beneficial to conduct comparative studies across different fish species and environmental conditions to determine the broad applicability of *Gongronema latifolium* in aquaculture. This could help optimize the use of this system in diverse aquaculture systems.
- **Integrated approaches:** Considering the potential synergistic effects, future studies should explore the combination of *Gongronema latifolium* with other natural or synthetic treatments to enhance its efficacy. Such integrated approaches could lead to more comprehensive strategies for improving fish health and nutritional quality.
- **Field trials:** Field trials in commercial aquaculture settings are recommended to validate the findings of this study and assess the practical feasibility of using *Gongronema latifolium* ethanolic extract as a dietary supplement or treatment in fish farming.

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