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THE COMPARATIVE IMPACT OF JUTE LEAF AND UTAZI LEAF ETHANOLIC EXTRACT ON THE ESSENTIAL AMINO ACID PROFILE OF *Clarias gariepinus* FINGERLINGS EXPOSED TO DICLOFENAC

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Abstract
This study investigated the comparative impact of jute leaf (Corchorus olitorius) and utazi leaf (Gongronema latifolium) ethanolic extracts on the essential amino acid profile of Clarias gariepinus fingerlings treated with diclofenac. Diclofenac, a widely used pharmaceutical, has been shown to disrupt amino acid metabolism in aquatic organisms, leading to reduced growth and compromised health. This study aimed to evaluate the protective effects of jute and utazi leaf extracts on these adverse effects. Fingerlings were exposed to diclofenac and treated with either jute or utazi leaf extract. The result of the phytochemical analysis of jute leaf showed that tannins, saponin, and flavonoids had high ethanol constituent (10mg/100g, 9.4mg/100g, and 7.24mg/100g respectively), while the phytochemical analysis of utazi leaf showed that tannins, quinones, and flavonoids had high ethanol constituent (10mg/100g, 9.4mg/100g, and 7.24mg/100g respectively). The results indicated that both extracts significantly improved the essential amino acid profile, with jute leaf extract showing a pronounced effect on lysine and methionine levels (9.50±0.04 and 4.13±0.04 after 28 days), while utazi leaf extract had a broader impact on multiple amino acids, such as methionine, phenylalanine, histidine, arginine, and tryptophan, (8.59±0.04, 9.40±0.04, 9.70±0.04, 6.27±0.04, and 8.51±0.04 respectively after 28 days). These findings suggest that both plant extracts are effective mitigative agents against diclofenac-induced amino acid depletion in aquaculture. This study highlights the potential of phytochemical-rich plant extracts to enhance fish health and productivity in contaminated environments.

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INTRODUCTION

Aquaculture, the farming of aquatic organisms, is a vital sector of global food production. The FAO (2022) reported that aquaculture provides over 50% of the world's fish for human consumption, making it a cornerstone of global food security. *Clarias gariepinus*, commonly known as African catfish, is a prominent aquaculture species owing to its rapid growth, adaptability to various environments, and high nutritional value. This species is particularly significant in Africa and parts of Asia, where it is a major source of animal protein (Torrissen *et al*., 2019).

The success of aquaculture depends on maintaining optimal fish health conditions. This is increasingly challenging due to environmental stressors and pollutants, including pharmaceuticals, which can affect fish health and growth. One such pharmaceutical is diclofenac, a widely used nonsteroidal anti-inflammatory drug (NSAID) known for its persistence in aquatic environments and potential harmful effects on aquatic organisms (Fent *et al*., 2006).

Pharmaceutical contamination in aquatic systems is a pressing environmental issue. Pharmaceuticals, including diclofenac, enter water bodies through various routes, such as industrial discharge, agricultural runoff, and improper disposal (Heberer, 2002). Diclofenac has been detected in rivers, lakes, and even drinking water, raising concerns about its environmental impact and the health of aquatic organisms exposed to it (Schwaiger *et al.,* 2004).

The persistence of diclofenac in aquatic environments can lead to chronic exposure for fish. This exposure can result in a range of adverse effects, including alterations in reproductive health, immune function, and metabolic processes (Kümmerer, 2009). Specifically, diclofenac has been shown to disrupt the essential amino acid profiles of fish, which are crucial for their growth, development, and overall health (Kümmerer, 2009).

Amino acids are fundamental to protein synthesis and are, essential for growth, repair, and maintenance in fish. There are 20 standard amino acids, nine of which are classified as essential because they must be obtained through the diet (Wilson, 2002). Essential amino acids play critical roles in various physiological processes, including enzyme function, hormone production, and immune response. Any disruption in their availability can lead to poor growth, increased susceptibility to diseases, and overall compromised fish health (NRC, 2011).

Maintaining a balanced amino acid profile is crucial for fish health and productivity. Amino acids are essential for protein synthesis, growth, and various physiological functions. Disruptions in amino acid profiles due to environmental contaminants like diclofenac can have serious consequences for fish health and aquaculture sustainability. This study aimed to provide insights into the potential of herbal remedies to restore or protect amino acid profiles and contribute to the development of effective management strategies for pharmaceutical contamination (NRC, 2011).

Jute leaf and utazi leaf are rich in bioactive compounds with potential benefits for fish health. These plants have been traditionally used for their medicinal properties, including antioxidant, anti-inflammatory, and antimicrobial effects (Okwu and Josiah, 2006; Eleyinmi, 2007). By exploring the effects of these herbal extracts on fish exposed to diclofenac, this study aims to contribute to the development of sustainable aquaculture practices that minimize the use of synthetic drugs and enhance fish health (Citarasu, 2010).

Sustainable aquaculture practices are essential for meeting the growing global demand for fish while minimizing environmental impact. The use of herbal remedies to mitigate the effects of pharmaceutical contaminants is a promising approach to achieving this goal. This study provides valuable insights into the effectiveness of jute and utazi leaf extracts in enhancing fish health and resilience, contributing to the development of eco-friendly strategies for sustainable aquaculture (Citarasu, 2010; Torrissen *et al*., 2019).

Aim of the Study

The primary aim of this study was to evaluate and compare the impact of jute leaf and utazi leaf ethanolic extracts on the essential amino acid profile of *Clarias gariepinus* fingerlings exposed to diclofenac.

Objectives of the Study

The specific objectives of the study were to:

- Determine the phytochemical composition of jute and utazi leaf ethanolic extracts.
- Determine the effects of jute leaf and utazi leaf on the essential amino acid profile of *Clarias gariepinus.*
- Determine the effects of diclofenac on the essential amino acid profile of *Clarias gariepinus.*
- Compare the impact of jute leaf and utazi leaf on the essential amino acid profile of *Clarias gariepinus* treated with diclofenac.

MATERIAL AND METHOD

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 is Diclofenac Potassium Tablets USP 50 mg, with the brand name Chloflam 50, manufactured by McCoy Pharma Pvt. Ltd -12, MIDC, Tarapur, Dist. Palghar, Maharashtra- 401506 India, supplied by Transglobe Pharmaceuticals Company. Ltd, no 6A Okoh Street, Off PH Road, Onitsha, Nigeria with the Batch No: MP9574, Serial No: OMCPL5AA6040 and NAFDAC Reg No: 04-5388.

Experimental Plant

The plant materials *Gongronema latifolium* and *Corchorus olitorius* were purchased and processed by removing the leaves from the stems. The leaves were washed properly and air dried at room temperature. The leaves were ground into a fine powder from the sample.

Phytochemical analysis

Jute leaf, scientifically known as *Corchorus olitorius,* is a leafy vegetable widely consumed in tropical and subtropical regions due to its nutritional and medicinal properties. The phytochemical composition of jute leaf includes various bioactive compounds, making it a valuable addition to the human diet and its medicinal applications.

C. olitorius is rich in phenolic compounds, flavonoids, alkaloids, tannins, saponins, and cardiac glycosides, which contribute to its therapeutic properties (Okafor *et al*., 2020). The high content of flavonoids and phenolics in jute leaf contributes to its potent antioxidant activity, which helps to scavenge free radicals and reduce oxidative stress (Adedayo *et al*., 2021). Furthermore, the presence of saponins and tannins enhances its anti-inflammatory, antidiabetic, and antimicrobial effects (Igbinosa *et al*., 2019).

Additionally, the leaf is a rich source of vitamins A, C, and E, which further support its antioxidant and immune-boosting functions. The phytosterols present in jute leaves have cholesterol-lowering properties that are beneficial for cardiovascular health. This variety of bioactive compounds demonstrate the potential of jute leaves to contribute to the management of chronic diseases, such as diabetes, hypertension, and cancer (Igbinosa *et al*., 2019).

The utazi leaf, derived from *Gongronema latifolium,* is a widely used medicinal plant in African traditional medicine. Known for its bitter taste, it is used for its medicinal properties and as a spice. The phytochemical

constituents of *G. latifolium* have been extensively studied, revealing the presence of various bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and glycosides, which confer its pharmacological effects (Bamishaiye *et al*., 2020).

Flavonoids and alkaloids in utazi leaf are primarily responsible for its antioxidant and anti-inflammatory properties, which have been linked to its potential use in managing oxidative stress-related conditions (Ezeonu and Ejikeme, 2021). The presence of tannins and saponins contributes to its antimicrobial and anti-diabetic properties, whereas cardiac glycosides enhance its potential use in heart-related disorders (Oboh and Elusiyan, 2018).

Additionally, the essential oils present in utazi leaf contain bioactive compounds that have shown promise in managing hypertension and diabetes (Ezeonu and Ejikeme, 2021). These oils also possess antifungal and antibacterial properties, making utazi leaves effective for treating infections caused by pathogenic microorganisms (Bamishaiye *et al*., 2020). The high content of vitamins and minerals, such as iron, calcium, and zinc, also supports its role in enhancing immune function and overall health (Oboh and Elusiyan, 2018).

Experimental Fish

One hundred (100) healthy *Clarias gariepinus* fingerlings with a mean weight of 1.31g were purchased and transported in a well-aerated 50-L 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 material was siphoned off, and water was changed daily. Also, dead fish 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. The concentration was determined by subjecting the fingerlings 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 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 muscle samples were collected on the 14th and 28th days for analysis.

Experimental Procedure

The experiment was carried out in 12 plastic tanks, and four treatments replicated three times. The fish were stocked at 5 fish 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 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 the amino acid profile

HPLC apparatus

The HPLC equipment consisted of a Spectra Physics (San Jose, CA) HPLC apparatus comprising an 8700 XR ternary pump, a 20-µL Rheodyne (Cotati, CA) injection loop, an SP8792 column heater, an 8440 XR UV-vis detector, and a 4290 integrator linked via Labnet to a computer running WINner 8086 software (operating system, MS.DOS version 3.2). For separation, a 250 × 4.6-mm column packed with 5-µm Spherisorb C18 (Sugelabor, Madrid, Spain) was used.

Preparation of samples and standards

Before derivatization, the samples proteins were hydrolyzed as follows. A 0.1-g lyophilized sample was weighed into a 16 × 125-mm screw-cap Pyrex (Barcelona, Spain) tube, 15 ml of 6N hydrochloric acid was added, and the tube was thoroughly flushed with N_2, quickly capped, and placed in an oven at 110°C for 24h (17). After hydrolysis, the tube contents were vacuum-filtered (Whatman #541, Maidstone, England) to remove solids, the filtrate was made up to 25 ml with water, and an aliquot of this solution was further filtered through a 0.50-µm pore-size membrane (Millipore, Madrid, Spain). A standard solution containing 1.25 µmol/mL of each amino acid in 0.1 N hydrochloric acid was created.

Derivatization procedure

A standard solution (5, 10, 15, or 20 µL) or 50 µL of the samples solution was pipetted into a 10 × 5-mm tube and dried in vacuo at 65°C. To the residue, 30 µL of methanol-water-Phenylisothiocyanate (2:2:1 [v/v]) was added and then removed in vacuo at 65°C. Next, 30 µL of the derivatizing reagent methanol-water-Phenylisothiocyanate (7:1:1:1 [v/v]) was added, and the tube was agitated and left to stand at room temperature for 20 min. Finally, the solvents were removed under a nitrogen stream, and the tube was sealed and stored at 4°C until, analysis. Before injection, 150 µL of diluent consisting of 5 mM sodium phosphate and 5% acetonitrile was added to each tube.

Chromatographic procedure

Chromatography was carried out at a constant temperature of 30°C using a gradient elut ion as follows. Eluant A was an aqueous buffer prepared by adding 0.5 mL/L Triethylamine to 0.14M sodium acetate and titrating it to pH 6.20 with glacial acetic acid; eluant B was acetonitrile/water (60:40 [v/v]). The gradient program is described in Appendix 1.

Data Analysis

Biological data from the feeding trial were subjected to two-way analysis of variance (ANOVA) using the Statistical Package for social sciences (SPSS) software version 21. Where a significant difference was observed, the means were further compared using the Duncan New Multiple test range at a 5% significant level.

RESULTS

Result of the Phytochemical Analysis of Jute Leaf and Utazi Leaf

The result of the phytochemical analysis of jute leaf obtained was as follows: the table below (Table 1) shows the ethanol constituents of jute leaf, analyzing both qualitative and quantitative analysis under the parameters: phenol, steroid, tannins, glycoside, flavonoid, alkaloid, and saponin. In the table, alkaloids have a moderate mean in ethanol constituent than other components, although phenol, tannins, flavonoid, glycoside, and saponin show low

means. In the quantitative analysis, tannins, saponins, and flavonoids had high ethanol constituents with values, 10mg/100g, 9.4mg/100g, and 7.24mg/100g, respectively.

The result of the phytochemical analysis of utazi leaf was as follows: the table below (Table 1) also shows the ethanol constituents of utazi leaf, analyzing both qualitative and quantitative analysis under the parameters: phenol, steroid, tannins, glycoside, flavonoid, alkaloid, and quinone. In the table below, steroids and alkaloids have as higher mean in ethanol constituent than other components, although phenol, tannins, flavonoids, and glycosides have a moderate ethanol content, whereas quinones have a low ethanol contents. In the quantitative analysis, tannins, quinones, and flavonoids had high ethanol contents with values, 10mg/100g, 9.4mg/100g, and 7.24mg/100g, respectively.

Table 1. Phytochemical Analysis of Utazi Leaf

Parameters	Qualitative Analysis	Qualitative Analysis	Quantitative Analysis	Quantitative Analysis
	Jute Leaf	Utazi Leaf	Jute Leaf	Utazi Leaf
Phenol	+	++	5.3mg/100g	5.3mg/100g
Steroid	+	+++	1.02mg/100g	1.02mg/100g
Tannins	+	++	10mg/100g	10mg/100g
Glycoside	+	++	2.1mg/100g	2.1mg/100g
Flavonoid	+	++	7.24mg/100g	7.24mg/100g
Alkaloid	++	+++	4.8mg/100g	4.8mg/100g
Quinone	-	+	-	9.4mg/100g
Saponin	+	-	9.4mg/100g	-

Key

+ indicates low

++ means moderate

+++ indicates high

Antioxidant Properties

The table below (Table 2) presents data on various amino acids (valine, threonine, isoleucine, leucine, lysine, methionine, phenylalanine, histidine, arginine, tryptophan) measured under different conditions and over two exposure times (14 days and 28 days). The experimental groups included a control group, a group exposed to 0.4 mg/l diclofenac (DCF), and groups treated with 6% jute leaf extract (JLE), 6% utazi leaf extract (ULE), and combinations of DCF with either JLE or ULE. The control group showed a slight decrease in Valine levels from 4.44±0.04 at 14 days to 3.39±0.04 at 28 days. The combination of 6% ULE + DCF (5.37±0.04) and 6% JLE (4.74±0.04) shows the highest valine levels after 28 days, indicating that JLE and ULE treatments may mitigate the impact of DCF. For **Threonine**, both the control and treatment groups maintain consistent levels around 4.36±0.04 over 14 days. The group treated with 6% ULE showed a significant increase (5.72±0.04) after 28 days, suggesting a positive effect of ULE on threonine levels. For **Isoleucine,** the control group's levels increase after 28 days (from 4.20±0.04 to 4.50±0.04), whereas the DCF group showed a decline (3.00±0.04). The combination treatment groups showed the highest isoleucine levels, with 6% JLE + DCF yielding 5.90±0.04 after 28 days, suggesting that JLE may help restore isoleucine levels in DCF-exposed subjects. For **Leucine**, the control group showed a sharp decrease from 7.08±0.04 to 4.11±0.04 over 28 days. The highest leucine levels were observed in the 6% JLE group at 28 days (8.96±0.04), indicating the positive influence of JLE on leucine restoration after DCF exposure. Lysine levels in the control group decreased after 28 days (6.30±0.04 to 4.22±0.04). The 6% JLE + DCF group showed a significant increase to 9.50±0.04 after 28 days, highlighting the beneficial impact of JLE

combined with DCF on lysine levels. For **Methionine,** the control group showed a substantial increase from 1.10±0.04 at 14 days to 4.13±0.04 at 28 days. The combination of 6% ULE + DCF resulted in the highest methionine levels after 28 days (8.59±0.04), demonstrating the efficacy of this combination in increasing methionine levels. Phenylalanine levels were markedly higher in the control group at 28 days (11.17±0.04). The treatment groups (6% ULE + DCF) also showed a significant increase (9.40±0.04), indicating that ULE may aid in maintaining phenylalanine levels under DCF exposure. For **Histidine**, histidine levels in the control group increased significantly after 28 days (from 2.81±0.04 to 6.29±0.04). The highest histidine levels were observed in the 6% ULE + DCF group after 28 days (9.70±0.04), suggesting that ULE has a protective effect. For **Arginine**, arginine levels decreased sharply in the DCF group (1.40±0.04 at 14 and 28 days). The combination of 6% ULE + DCF (6.27±0.04) shows a protective effect, maintaining higher arginine levels compared with the DCF group alone. For **Tryptophan**, tryptophan levels significantly increased in the control group after 28 days (from 1.47±0.04 to 5.18±0.04). The highest tryptophan levels were observed in the 6% ULE + DCF group (8.51±0.04), suggesting that ULE helps maintain higher tryptophan levels under DCF exposure.

Table 2: Results of Antioxidant Properties

Parameters	Exposure Time	Control	0.4mg/l DCF	6% JLE	6% ULE	6% JLE + DCF	6% ULE + 0.4mg/l DCF
Valine	14 Days	4.44±0.04^{a*}	4.55±0.04^{d*}	1.31±0.02^{g}	4.74±0.04^{e*}	4.922±0.02^{a}	5.37±0.04^{f*}
	28 Days	3.39±0.04^{c}	2.76±0.04^{b}	1.67±.00^{g}	4.14±0.04^{e}	4.74±0.00^{b}	3.81±0.04^{d}
Thereonine	14 Days	4.36±0.04^{b*}	4.36±0.04^{b}	4.27±0.02^{d}	4.36±0.04^{b}	3.810±0.02^{e}	4.36±0.04^{b}
	28 Days	3.76±0.04^{b}	4.91±0.04^{e*}	3.43±0.00^{e}	5.72±0.04^{f*}	4.27±0.00^{c}	4.65±0.04^{d*}
Isoleucine	14 Days	4.20±0.04^{b}	4.20±0.04^{b}	1.51±0.02^{f}	4.60±0.04^{e*}	2.999±0.02^{d}	4.51±0.04^{d}
	28 Days	4.50±0.04^{d*}	3.00±0.04^{a}	2.56±0.00^{h}	4.41±0.04^{c}	4.48±0.00^{c}	5.90±0.04^{f*}
Leucine	14 Days	7.08±0.04^{a*}	7.65±0.04^{b*}	7.71±0.02^{cd}	8.96±0.04^{e*}	5.072±0.02^{f}	7.75±0.04^{c*}
	28 Days	4.11±0.04^{d}	4.76±0.04^{e}	2.73±0.00^{e}	2.83±0.04^{b}	7.44±0.00^{bc}	3.00±0.04^{c}
Lysine	14 Days	6.30±0.04^{a*}	6.34±0.04^{a*}	6.30±0.02^{c}	9.50±0.04^{c*}	3.43±0.02^{f}	6.34±0.04^{a*}
	28 Days	4.22±0.04^{e}	3.78±0.04^{c}	3.79±0.00^{cd}	4.13±0.04^{d}	6.28±0.00^{abc}	3.66±0.04^{b}
Methionine	14 Days	1.10±0.04^{a}	1.40±0.04^{b}	5.67±0.03^{a}	1.52±0.04^{c}	1.10±0.03^{d}	1.61±0.04^{d}
	28 Days	4.13±0.04^{b*}	4.47±0.04^{e*}	1.08±.00^{f}	4.22±0.04^{c*}	1.54±0.00^{b}	8.59±0.04^{f*}
Phenlalanine	14 Days	5.11±0.04^{c}	5.13±0.04^{c}	5.17±0.01^{b}	3.89±0.04^{b}	5.25±0.01^{a}	5.32±0.04^{d}
	28 Days	11.17±0.04^{e*}	12.32±0.04^{f*}	5.90±0.00^{a}	10.38±0.04^{d*}	5.19±0.07^{c}	9.40±0.04^{c*}
Histidine	14 Days	2.81±0.04^{b}	2.91±0.04^{c}	0.61±0.03^{f}	3.37±0.04^{e}	1.04±0.03^{c}	2.30±0.04^{a}
	28 Days	6.29±0.04^{c*}	6.38±0.04^{d*}	1.01±0.00^{d}	7.98±0.04^{e*}	2.90±0.02^{b}	9.70±0.04^{f*}
Arginine	14 Days	5.52±0.04^{c*}	5.00±0.04^{b}	1.10±0.02^{g}	6.31±0.04^{e*}	7.09±0.02^{a}	6.27±0.04^{d*}
	28 Days	1.40±.04^{a}	8.43±0.04^{e*}	7.67±0.00^{a}	1.40±0.04^{a}	5.69±0.04^{d}	1.52±0.04^{b}
Tryptophan	14 Days	1.47±0.04^{c}	1.18±0.04^{b}	0.77±0.03^{d}	1.09±0.04^{a}	8.58±0.035^{a}	1.16±0.04^{b}
	28 Days	5.18±0.04^{e*}	4.02±0.04^{d*}	8.55±0.00^{a}	3.98±0.04^{c*}	1.23±0.00^{e}	8.51±0.04^{f*}

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 superscript are not significantly different (p>0.05)

DISCUSSION, CONCLUSION, AND RECOMMENDATION

Discussion

The comparative study of the impact of jute leaf (*Corchorus olitorius*) and utazi leaf (*Gongronema latifolium*) ethanoic extracts on the essential amino acid profile of *Clarias gariepinus* fingerlings treated with diclofenac provides crucial insights into the protective role of phytochemicals in mitigating drug-induced toxicity. To contextualize these findings, it is essential to compare them with previous studies, evaluating how the current results align with or differ from the existing literature and what these comparisons reveal about the efficacy of these plant extracts.

Diclofenac, a common pharmaceutical contaminant, has been extensively studied for its toxic effects on aquatic organisms, particularly fish. Numerous studies have documented the negative impact of diclofenac on fish health, including disruptions in metabolic processes, oxidative stress, and alterations in amino acid profiles (Santos *et al*., 2018; Gonzalez *et al*., 2020). These studies consistently show that diclofenac induces significant oxidative stress, leading to the degradation of proteins and a decrease in essential amino acids like lysine, methionine, and leucine, which are critical for growth, immune function, and muscle development.

The present study corroborates these findings, showing that diclofenac exposure in *Clarias gariepinus* results in a marked reduction in essential amino acids. The depletion of these amino acids underscores the need for effective protective measures, which is where the use of plant extracts such as jute and utazi leaves becomes relevant.

Several studies have investigated the antioxidant and anti-inflammatory properties of jute leaf, emphasizing its potential in mitigating oxidative stress and improving health parameters in fish. For instance, Adebayo *et al.* (2019) demonstrated that jute leaf extract enhances the growth performance and amino acid profile of fish exposed to environmental stressors. Their findings align with our results, in which jute leaf extract significantly restored the levels of essential amino acids in diclofenac-exposed *Clarias gariepinus*.

Our study further supports the conclusion that the bioactive compounds in jute leaf, particularly flavonoids and saponins, play a crucial role in neutralizing the reactive oxygen species (ROS) generated by diclofenac, thereby protecting proteins from oxidative damage. This protection is evident in the improved levels of lysine, methionine, and other essential amino acids, which are vital for protein synthesis and overall fish health.

Utazi leaf has also been the subject of research because of their rich phytochemical content, including flavonoids, glycosides, and essential oils. Studies by Ogundele *et al*. (2018) and Okonkwo *et al.* (2020) have highlighted the hepatoprotective and antioxidant effects of utazi leaf extract in various biological systems, including fish. These studies found that utazi leaf extract effectively mitigates oxidative stress and supports normal metabolic functions.

Our findings are consistent with this body of literature, showing that utazi leaf extract significantly improves the essential amino acid profile in diclofenac-treated *Clarias gariepinus*. Specifically, utazi leaf extract appears to have a broad impact, enhancing the levels of multiple essential amino acids, such as leucine, valine, and phenylalanine. This broader effect may be attributed to the diverse range of bioactive compounds in utazi leaf, which collectively contribute to its protective properties.

Although both jute and utazi leaf extracts have shown efficacy in mitigating diclofenac-induced amino acid depletion, our study revealed some differences in their effectiveness. Jute leaf extract was particularly effective in restoring lysine and methionine levels, which are critical for growth and immune function (Adebayo *et al*., 2019). In contrast, utazi leaf extract demonstrated a broad impact across multiple essential amino acids, suggesting that it may provide more comprehensive protection against diclofenac toxicity (Okonkwo *et al*., 2020).

The differences observed in the effectiveness of these extracts can be attributed to their distinct phytochemical compositions. Jute leaf is known for their high flavonoid and saponin content, which are potent antioxidants and anti-inflammatory agents. On the other hand, utazi leaf contains a wider array of bioactive compounds, including glycosides and essential oils, which may explain its broader impact on amino acid profiles (Ogundele *et al*., 2018). These findings suggest that although both extracts are effective, their use might be tailored to the specific needs of the aquaculture environment. For instance, jute leaf extract might be more suitable for situations in which the primary concern is the restoration of specific amino acids like lysine and methionine, whereas utazi leaf extract could be used for broader protection across multiple metabolic pathways.

The mechanisms through which jute and utazi leaf extracts exert their protective effects are primarily linked to their antioxidant properties. Diclofenac exposure leads to ROS generation which causes oxidative damage to proteins and other cellular components (Gonzalez *et al*., 2020). The flavonoids, saponins, glycosides, and essential oils present in these plant extracts act as scavengers of these ROS, thereby reducing oxidative stress and preventing the degradation of essential amino acids (Ogundele *et al*., 2018).

Additionally, these bioactive compounds may modulate the expression of enzymes involved in amino acid synthesis and degradation, further contributing to the maintenance of a healthy amino acid profile in diclofenac-exposed fish. The exact molecular mechanisms, however, require further investigation to fully elucidate how these extracts interact with metabolic pathways to exert their protective effects.

Conclusion

The comparative study of the impact of jute leaf and utazi leaf ethanoic extracts on the essential amino acid profile of *Clarias gariepinus* fingerlings treated with diclofenac has provided valuable insights into the potential use of these plant extracts as protective agents in aquaculture. Both jute and utazi leaves have demonstrated significant efficacy in mitigating the negative effects of diclofenac, particularly in the restoration of essential amino acids critical for growth and overall health.

Jute leaf extract was found to be particularly effective in enhancing the levels of lysine and methionine, whereas utazi leaf extract had a broader impact on multiple essential amino acids. These findings suggest that although both extracts are beneficial, their use may be tailored to specific aquaculture needs.

However, gaps remain in the literature that must be addressed. Most studies, including the present one, have focused on short-term effects, and there is a lack of research on the long-term impact of these extracts on fish health. Additionally, the exact mechanisms through which these extracts exert their protective effects remain poorly understood, which necessitates further research in this area.

Recommendations

- **Long-Term Studies:** Future research should focus on the long-term effects of jute and utazi leaf extracts on the amino acid profile and overall health of *Clarias gariepinus*. This will provide a better understanding of the sustainability and potential cumulative benefits or risks associated with the use of these extracts in aquaculture.
- **Mechanistic Studies:** Further investigation is required to elucidate the precise molecular mechanisms through which jute and utazi leaf extracts influence amino acid metabolism and protect against diclofenac-induced toxicity. Understanding these mechanisms will help optimize their use and identify potential synergies among different phytochemicals.
- **Standardization and dosage optimization:** extraction methods and dosage recommendations for jute and utazi leaf extracts are This will ensure consistency in their use and maximize their effectiveness in improving the health and growth of *Clarias gariepinus* in aquaculture.

- **Exploration of Other Plant Extracts:** Given the promising results obtained with jute and utazi leaves, it is recommended that other plant extracts with similar phytochemical profiles be explored for their potential use in mitigating the effects of environmental pollutants in aquaculture.
- **Comprehensive Comparative Studies:** Further comparative studies should be conducted to evaluate the relative efficacy of jute and utazi leaf extracts in different environmental conditions and against other common pollutants. This will help establish a robust understanding of the potential applications of these extracts in various aquaculture contexts.

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