

Dietary Methanolic Extracts of *Justicia carnea* and *Ocimum gratissimum* on Growth Response, Haematological Indices and Serum Biochemical Profiles of Female African Catfish (*Clarias gariepinus*)

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Abstract

A total of 300 juvenile female African catfish sourced from Tolu's Farms, Enugu State, were used in this study. Upon arrival, the fish were acclimatized for one week and then randomly allocated to four treatment groups following a 2x2 factorial arrangement in completely randomized design (CRD), with each treatment replicated four times. The fish were fed diets containing varying levels of *Justicia carnea* and *Ocimum gratissimum* leaf extracts. Fresh leaves of *Justicia carnea* (JC) and *Ocimum gratissimum* (OG) were harvested from a greenhouse farm, thoroughly cleaned, air-dried, and milled into powder. The powdered samples were subjected to methanolic extraction at the Biochemistry Laboratory of the Department of Animal Science. Twenty-five grams (25 g) of each extract were diluted in 50 cl of water. Subsequently, 1 kg of fish feed was separately and uniformly mixed with 50 ml of JC extract and 25 ml of OG extract. Data obtained were analyzed statistically using IBM SPSS statistics version 23. The results obtained showed that the inclusion of the plant extracts significantly enhanced the growth performance of the fish. Hematological parameters indicated that treatments T₃ and T₄ recorded the highest packed cell volume (PCV), red blood cell (RBC) counts, and hemoglobin (Hb) concentrations, which were significantly higher ($p < 0.05$) than those of the control group (T₁). Similarly, serum biochemical analysis revealed significantly lower ($p < 0.05$) low-density lipoprotein (LDL) levels in T₃ and T₄ compared with T₁. In contrast, the control group (T₁) exhibited higher levels of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) relative to the treatment groups. Furthermore, total cholesterol and creatinine levels were significantly reduced in fish from T₃ and T₄ compared with the control. These findings indicate that dietary supplementation with *Justicia carnea* and *Ocimum gratissimum* leaf extracts enhances growth performance and improves hematological and serum biochemical indices, thereby promoting the safety and quality of fish meat. In conclusion, these plant extracts showed considerable potential as natural dietary supplements for use in aquaculture.

Keywords: Methanolic extract; Serum; Haematology; African catfish; Scent leaf; Brazilian plume

1. Introduction

Fish farming has emerged as the fastest-growing sector within global agricultural enterprises (Kaleem and Sabi, 2021). This rapid expansion is attributed to several factors, including the species' fast growth rate, resilience to adverse environmental conditions, ability to utilize a variety of agricultural by-products as feed, ease of fingerling acquisition, high fecundity, efficient feed conversion ratio and strong consumer demand. These advantages have contributed significantly to the steady growth of Nigeria's catfish industry.

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Despite these potentials, aquaculture in Nigeria faces several challenges that hinder its development, chief among them being the high cost of fish feed. Additionally, the conventional reliance on antibiotics and chemical treatments in aquaculture has come under increasing scrutiny due to the potential for promoting antibiotic-resistant bacteria, environmental pollution, and the accumulation of chemical residues in fish tissues (Suyamud *et al.*, 2024). These issues not only compromise fish health but also pose risks to consumers.

Consequently, in the management of aquaculture especially within intensive and semi-intensive systems there is growing interest in dietary supplementation with phytogenic compounds. These plant-based additives are gaining attention for their potential to enhance fish growth and productivity. The World Health Organization (2019) advocates the use of medicinal herbs and plants as alternatives to chemical inputs, reflecting a broader global movement toward natural, eco-friendly solutions. This paradigm shift from synthetic drugs to botanical products has sparked research into the use of plant extracts to improve animal health and performance. Among the promising herbs are *Justicia carnea* (commonly called "Britain plume") and *Ocimum gratissimum* (scent leaf).

Justicia carnea, also known as *Ewe Eje* in Yoruba and *Ogwu Obara* in Igbo, holds a prominent place in traditional medicine for its therapeutic benefits, including promoting blood formation, reducing inflammation, and relieving pain (Oloruntola *et al.*, 2022). The plant is not cost-effective, easily propagated vegetatively, and widely accessible. Nutritionally, it is rich in vitamins A, B1, B6, B9, B12, and E, as well as essential minerals such as iron, calcium, magnesium, zinc, and copper (Andrew *et al.*, 2024). Furthermore, it contains a wide range of bioactive phytochemicals that enhance its medicinal efficacy.

Similarly, *Ocimum gratissimum*, known as clove basil, African basil, or wild basil, is a perennial herb of the Lamiaceae family with a strong aromatic profile and substantial economic value. It is widely distributed across Africa, Asia, and South America (Ugbogu *et al.*, 2021). Frequently used as a culinary spice in fish, meat, soups, and stews, scent leaf also serves medicinal purposes in traditional healthcare systems. It is employed in the treatment of ailments such as fever, inflammation, anemia, diarrhea, and infections caused by fungi and bacteria (Shedoeva *et al.*, 2019).

Fish farming has been recognized as the fastest-growing segment of global agricultural businesses (Kaleem and Sabi, 2021). The reasons could be traced to its rapid growth rates, hardness to unfavourable conditions, feeds on wild varieties of agricultural by-products, easy procurement of fingerlings, high fecundity, high feed conversion ratio and high consumer preference. And this has led to gradual expansion of catfish industry in Nigeria.

However, aquaculture in Nigeria has its short comings that have militated against the growth and development of the industry such as quality and high cost of feeds. More so, the traditional use of antibiotics and other chemical treatments in aquaculture has been widely criticized due to the risks of developing antibiotic-resistant bacteria, environmental contamination, and residue accumulation in fish (Suyamud *et al.*, 2024). Some of which may influence the physiological status of the animals and health of the consumers.

Thus, in aquaculture management, particularly in intensive or semi-intensive systems, dietary phytogenic supplementation plays an essential role and holds promise for boosting fish production. World Health Organization (2019) encourages using of medicinal herbs and plants to substitute or minimize the use of chemicals through the global trend to go back to nature. This shift from synthetic drugs to plant-based products has fueled growing interest in using plant extracts to enhance animal growth and performance. Plants once overlooked are now being studied, evaluated, and developed due to their minimal side effects. Some of such plants are Britain plume (*Justicia carnea*) and scent leave (*Ocimum gratissimum* L.) among others.

Justicia carnea, known as *Ewe Eje* in Yoruba or *Ogwu Obara* in Igbo, has a rich history in traditional medicine. It is renowned for its ability to boost blood levels, reduce inflammation, and alleviate pain, among other benefits (Oloruntola, *et al.*, 2022). This plant is affordable and readily accessible as it undergoes vegetative propagation. Nutritionally, *J. carnea* is a rich source of vitamins A, B1, B6, B9, B12, and E, along with essential minerals such as iron, calcium, magnesium, zinc, and copper (Andrew *et al.*, 2024). In addition to its nutritional value, the plant has abundant beneficial phytochemicals that contribute to its medicinal properties (Andrew *et al.*, 2024).

Scent leaf (*Ocimum gratissimum*), on the other hand is commonly known as clove basil, African basil, or wild basil. It is a perennial herb with a strong aroma and significant commercial potential. Belonging to the Lamiaceae family, it is found across Africa, Asia, and South America (Ugbogu *et al.*, 2021). Scent leaf is often used as a spice for fish, meat, soups, and stews and is traditionally employed to treat conditions such as aches, fever, inflammation, anemia, diarrhea, fungal and bacterial infections (Shedoeva *et al.*, 2019).

2. Materials and methods

2.1. Location of study

The present experiment was carried out at the fishery unit of the farm operations, Faculty of Agriculture, University of Nigeria Nsukka. See the map and the arrow below.

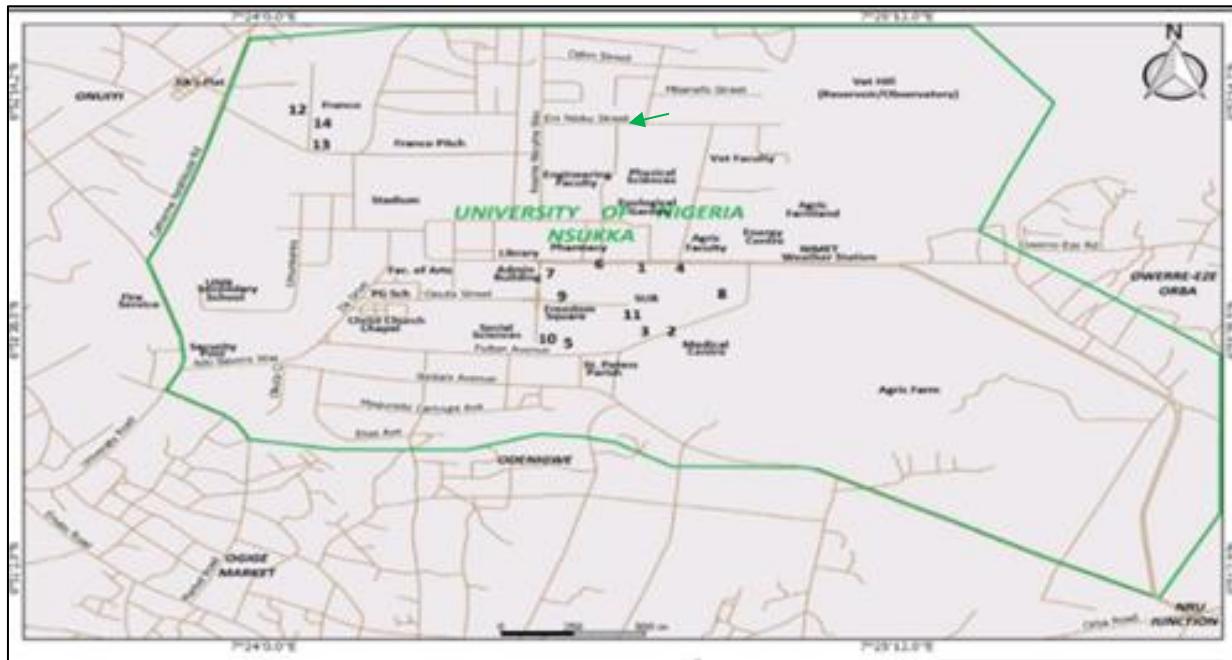


Figure 1 Map of the study area

2.2. Duration of study

This experiment lasted for a period of 8 weeks

2.3. Management of Experimental fish

A total of 300 juvenile African catfish (*Clarias gariepinus*), averaging 13.5 ± 0.82 g in weight, were obtained from Tolu farms in Enugu State Nigeria. On arrival, the fish were stocked at the Farm Operation unit of the Faculty of Agriculture, University of Nigeria, Nsukka and allowed to acclimatize for two weeks in one of the big reconstructed ponds.

The fish were randomly assigned to four treatment groups following a completely randomized design in a 2x2 factorial experiment. Each group consisted of three replicates, with 25 juveniles per replicate. To maintain water quality and ensure proper hygiene, the water was changed weekly. The fish were fed daily at a rate of 5% of their body weight.

Where;

T₁ (Control): Fish in this treatment was given 0 ml of *J. carnea* extract and 0 ml of *O. gratissimum* leaf extract

T₂: Fish in this treatment was given 50 ml of *J. carnea* extract and 0 ml of *O. gratissimum* leaf extract

T₃: Fish in this treatment was given 0 ml of *J. carnea* extract and 25 ml of *O. gratissimum* leaf extract

T₄: Fish in this treatment was given 50 ml of *J. carnea* extract and 25 ml of *O. gratissimum* leaf extract

Table 1 Experimental Layout

		<i>Ocimum gratissimum (OG)</i>	
<i>Justicia carnea (JC)</i>	<i>OG</i>	0ml	25ml
	<i>JC</i>	0 ml	0 ml + 0 ml
	50 ml	50 ml JC + 0 ml OG	50 ml JC + 25 ml OG

2.4. Procurement and Preparation of *J. carnea* and *O. gratissimum* Leaf Extract

The leaves of *Justicia carnea* and *Ocimum gratissimum* were collected from the Department of Crop Science Teaching and Research farm UNN. They were thoroughly washed, air-dried for one week, and ground into fine powder. Methanol was used to extract the active components from the powdered leaves, with the extraction process carried out in the Laboratory Unit of the Department of Animal Science, UNN. For the extraction, 3000 ml of *Justicia carnea* leaf powder and 1500 ml of *Ocimum gratissimum* leaf powder were soaked in 300 g and 150 g of methanol respectively for three days at room temperature. The extraction was carried out using the method as described by Sofowora (2008). The mixture of the measured methanol and the plant leaf meals were kept in sealed amber glass containers and allowed to stand for 72 h at room temperature with intermittent agitation to enhance extraction efficiency. The resulting mixture was filtered initially through muslin cloth and subsequently through Whatman No. 1 filter paper to remove plant residues.

The combined filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi or equivalent) set at a water bath temperature of 40–45 °C, rotation speed of 80–120 rpm, and vacuum pressure adjusted to ensure efficient solvent evaporation without thermal degradation of bioactive compounds. Methanol was completely removed to obtain a viscous crude extract. The concentrated extract was further dried to constant weight in a vacuum desiccator. Extraction yield was calculated as the percentage ratio of the weight of the dried crude extract to the initial dry weight of the plant material

2.5. Experimental Ponds

Fifteen ponds measuring 2 x 3.0 x 1.8 m. each were used for the experiment. The ponds were first cleaned, disinfected with a quick lime at the rate of 750 to 1250 kg/ha and allowed to dry for one week. Afterwards the ponds were washed with clean water and fertilized by filling 25 kg bag of poultry manure each for every pond. The bags of manure were allowed to float on each pond for the period of one month. At the end of fertilization, the ponds were refreshed and fingerlings stocked.

2.6. Weighing of Fish

The fish were weighed using an electronic sensitive weighing balance (OHAUS LS-400 g model) prior to the commencement of the experiment and subsequently on a weekly basis throughout the experimental period. Fingerlings from all replicates in each treatment group were harvested weekly and weighed collectively. At the end of the experiment, all surviving fingerlings from each treatment were individually weighed after a 24-hour feed evacuation period (starvation) to obtain accurate final body weights and mean final weight.

2.7. Growth Parameters

Growth parameters were evaluated following the method described by Olvera-Novoa *et al.* (1990) using the following indices:

Feed Conversion Ratio (FCR)

Feed conversion ratio was determined as an index of feed utilization efficiency:

$$FCR = \frac{\text{Weight of feed given}}{\text{Weight gain of fish}}$$

Feed Intake

Feed intake was calculated as the sum of the daily mean feed consumed by fish in each treatment group over the entire experimental period.

Weight Gain

Weight gain was determined as the difference between the final and initial body weights:

$$\text{Weight Gain (g)} = \text{Final body weight (g)} - \text{Initial body weight (g)}$$

Specific Growth Rate (SGR, %/day)

Specific growth rate was calculated following Brown (1975):

This was the relationship of difference in weight of the fish within the experimental period and was calculated thus;

$$SGR = \frac{\log W_2 - \log W_1}{T_2 - T_1} \times \frac{1}{100}$$

Where W_2 = weight at time T_2 (days); W_1 = weight at time T_1 (days) (Brown, 1975).

Condition Factor (K)

The condition factor of *Clarias gariepinus* in the different treatments was calculated according to Adikwu (1992) as:

$$K = \frac{100W}{L^3}$$

Where W = Weight of fish and L = total length of fish

Length Measurements

Total length and standard length of juvenile *Clarias gariepinus* in the different treatments were measured using a fish measuring board and recorded to the nearest 0.01 cm

2.8. Haematological/ Biochemical Determination

Haematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration (Hb), packed cell volume (PCV) were determined at the end of the feeding trial following the methods described by Baker and Silverton (1985).

Fish were individually restrained manually, and blood samples (3ml each for haematology and serum biochemical indices) were collected from a total of 48 fish (three fish per replicate) via the caudal vein using a heparinized plastic syringe fitted with a 21-gauge hypodermic needle. For haematological analysis, the collected blood samples were immediately transferred into bottles containing disodium ethylene-diamine tetra-acetic acid (EDTA) as an anticoagulant and preserved for subsequent haematological analyses. In serum biochemical analysis, 3mls of blood samples were used for the analysis with EDTA. The following liver enzymes and lipid profiles were determined using the methods as described by Bahman, *et al.* (2011) alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol and triglycerides (TAG) and Creatinine.

Table 2 Proximate analysis of *Justicia carnea* leaf meal (percentage of dry samples)

Ingredients	<i>Justicia carnea</i>	<i>Ocimum gratissimum</i>
Crude Protein	27.00	26.52
Ether Extract	3.50	16.25
Crude Fibre	10.00	15.06
Ash	6.00	12.98
Nitrogen Free Extract	53.50	29.19
Moisture	10.93	7.46

Table 3 Results of Phytochemical Compositions of *Justicia carnea* and *Ocimum gratissimum* leaf extract

	<i>Justicia carnea</i>	<i>Ocimum gratissimum</i>
Phytochemicals	Methanolic extract	Methanolic extract
Alkaloids	++	++
Glycosides	++	+++
Saponin	++	+++

Flavonoid	+	++
Tannins	+++	++
Polyphenol	+++	++
Terpenoid	++	--

KEY: +++ = Highly present, ++ = Moderately present, + = Slightly present, -- = Not present

2.9. Data collection

Data was collected on the following parameters; the average initial body weight and length of the fingerlings at the commencement of the experiment. Similar record was taken on weekly basis. The total biomass of each treatment group was used to determine the quantity of feed given to the feed (5% biomass).

2.10. Experimental design

A 2 x 2 factorial arrangement in a completely Randomized design (CRD) was used for this experiment with statistical model given below;

$$Y_{ijk} = \mu + A_i + B_j + A_i B_j + \sum_{ijk}$$

Where Y_{ijk} represents the individual observation

μ = population mean or the overall mean

A_i = First Factor (*Justicia carnea*)

B_j = second factor (*Ocimum gratissimum*)

$A_i B_j$ = interaction effects of JC/OG

\sum_{ijk} = error

3. Results

Table 4 Effects of *Justicia carnea* and *Ocimum gratissimum* Leaf Extract on the Growth Indices of Female African Catfish (*Clarias gariepinus*)

JC/OG Levels (ml/kg)	T1(Control)	T2	T3	T4	
	0ml	50ml+ 0ml	0ml + 25ml	50ml+25ml	P. value
Initial Body Weight (g)	42.42	42.78	42.81	42.42	0.97
Final Body Weight (g)	312.06 ^c	354.06 ^a	346.44 ^b	336.66 ^b	0.00
Body Weight Gain (g)	269.58 ^c	311.28 ^a	303.63 ^a	292.24 ^b	0.00
Specific Growth Rate	0.97 ^b	1.03 ^a	1.02 ^a	1.00 ^a	0.02
Standard Length (cm)	30.63 ^a	30.76 ^a	30.50 ^a	27.55 ^b	0.03
Total Length (cm)	33.85	33.33	33.25	31.27	0.15
Feed Intake (g)	558.39 ^b	622.02 ^a	610.01 ^a	599.78 ^a	0.00
Feed Conversion Ratio	2.07 ^a	1.93 ^b	1.99 ^b	2.03 ^{ab}	0.04
Condition Factor (K)	1.08	1.23	1.22	1.44	0.07

Table 3 a, ab, b, c: rows with different superscript are significantly different ($p < 0.05$)

Table 4 presents the interaction effects of the methanolic leaf extract of *Justicia carnea* and *Ocimum gratissimum* at different levels on the growth performance of African Catfish (*Clarias gariepinus*). There were significant ($p < 0.05$) differences in the following parameters; final body weight, body weight gain, Specific growth rate, Standard length, Feed intake and Feed conversion ratio. In final body weight, T₂ had the highest final body weight of 354.06 g which differed ($p < 0.05$) significantly from the value of 346.44 g and 336.66 g obtained from T₃ and T₄ respectively. T₁ had the lowest final body weight of 312.06 g which differed ($p < 0.05$) significantly from the other treatment groups. In body weight gain, T₂ and T₃ had the highest value of 311.28 g and 303.63 g respectively which were themselves similar but different from T₄ while T₁ (Control) had the lowest body weight gain of 269.64 g which was significantly different from the other

treatment groups. In specific growth rate, T₂, T₃ and T₄ were statistically similar but differed (p < 0.05) significantly from T₁ (Control) which had the lowest value.

Standard length of the fish showed that T₁, T₂ and T₃ had the highest values which were themselves similar but differed (p < 0.05) from the T₄ which had the lowest value of 27.55 cm. Total length of the fish in this experiment followed the trend with the standard length. More so, in feed intake, T₂ and T₃ had the highest values which were themselves similar but differed significantly from the other treatment groups. However, in feed conversion ratio, T₂ and T₃ had the best feed conversion ratio of 1.93 and 1.99 respectively which were themselves similar but different from T₁ and T₄.

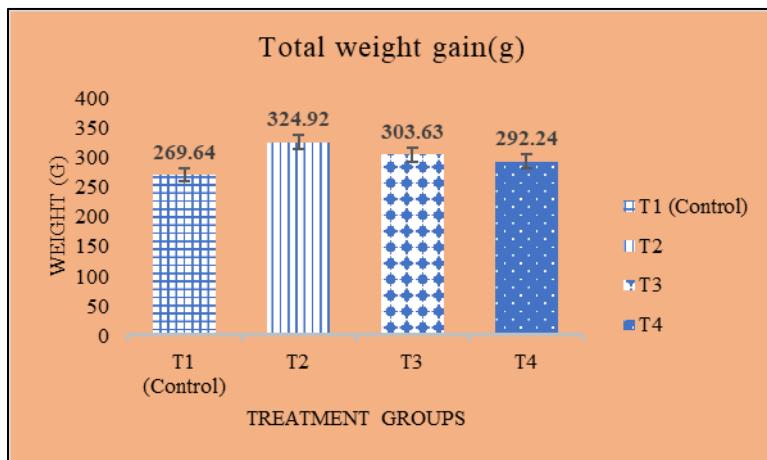


Figure 2 Graph representing the total weight gain of female African catfish fed dietary *J. carnea* and *O. gratissimum*. From the graph, T₁ (Control) had the lowest total weight gain while T₂ (50 ml of JC) had the highest total weight gain across the treatment means

Table 5 Effects of *Justicia carnea* and *Ocimum gratissimum* Leaf Extract on the Haematological Response of Female African Catfish (*Clarias gariepinus*)

	T1(Control)	T2	T3	T4	
JC/OG Levels (ml/kg)	0ml	0/50 ml	25/0ml	50/25 ml	P. Value
PCV (%)	33.50 ^c	33.50 ^c	35.0 ^b	37.0 ^a	0.00
RBC (g/dl)	9.71 ^b	10.20 ^b	10.80 ^a	10.81 ^a	0.01
WBC (mm ⁻³)	8700 ^a	7850 ^{ab}	7866.69 ^{ab}	7600 ^b	0.05
HB (g/dl)	7.75	8.25	8.83	8.85	0.07

a, b, c: row means with different super scripts are significantly different (p < 0.05)

Table 5, showed that there were significant (p < 0.05) differences in all the parameters analyzed except in Haemoglobin concentration. In Packed cell Volume (PCV), T₄ had the highest PCV value of 37.00 % which was significantly different from T₃ (35 %). T₁ and T₂ were themselves similar and significantly lower than the other treatment groups. In RBC, T₃ and T₄ had the highest values of 10.80 g/dl and 10.81 g/dl respectively which were themselves similar but differed significantly from T₁ (9.71 g/dl) and T₂ (10.20 g/dl) which were themselves similar. Haemoglobin concentration followed the similar trend with that of RBC. However, in WBC, T₁ had the highest value of 8700 mm⁻³ which significantly higher than T₂ and T₃ which were themselves similar. However, T₄ had the lowest value of 7600 mm⁻³ which was significantly (p < 0.05) different from the other treatment groups.

Table 6 Effect of *Justicia carnea* and *Ocimum gratissimum* Leaf Extract on the Serum Biochemical Indices of Female African Catfish (*Clarias gariepinus*)

JC/OG ml/kg	T1 (Control)	T2	T3	T4	P. value
ALT (IU/L)	28.00 ^a	24.00 ^b	21.50 ^c	23.50 ^b	0.01
AST (IU/L)	72.00 ^a	56.50 ^b	53.50 ^c	52.50 ^c	0.00
ALP (IU/L)	42.50 ^a	38.50 ^b	38.00 ^b	35.00 ^c	0.03
LDL (mg/dl)	24.50 ^a	21.50 ^b	21.50 ^b	19.50 ^c	0.02
HDL (mg/dl)	51.50 ^c	60.50 ^b	62.00 ^{ab}	63.50 ^a	0.00
Total Cholesterol (mg/dl)	95.58 ^a	93.00 ^b	82.00 ^c	82.50 ^c	0.00
Creatinine (mg/dl)	3.58 ^a	3.52 ^a	3.52 ^a	2.56 ^b	0.00

Key: a, ab, b, c = rows with different superscript are significantly different ($p < 0.05$); Alanine Aminotransferase (ALT); Aspartate Aminotransferase (AST); Alkaline Phosphatase (ALP); Low Density Lipoprotein (LDL); High-Density Lipoprotein (HDL)

The control group (T₁) had the highest ALT value 28.00 mg/dl which was significantly higher than the other treatment groups. While T₂ and T₄ had lower ALT values of 24.00 mg/dl and 23.50 mg/dl respectively which were significantly different from the control group. The group with 25 ml/kg *Ocimum gratissimum* (T₃) had the lowest ALT value (21.50) among all the treatments. The AST levels were also significantly different among the treatments. The control group (T₁) had the highest AST value (72.00), which was significantly higher than the other treatment groups. The groups with 25 ml/kg *Ocimum gratissimum* (T₃), and 50 ml/kg *Justicia carnea* + 25 ml/kg *Ocimum gratissimum* (T₄) had lower and statistically similar AST values (53.50, and 52.50, respectively). The ALP levels were significantly different among the treatments. The control group (T₁) had the highest ALP value (42.50), which was significantly higher than the other treatment groups. The group with 50 ml/kg *Justicia carnea* + 25 ml/kg *Ocimum gratissimum* (T₄) had the lowest ALP value (35.00) among all the treatments. The groups with 50 ml/kg JC + 0 ml/kg OG(T₂) and 0 ml *J. carnea* + 25 ml/kg *O. gratissimum* (T₃) had intermediate and statistically similar ALP values (38.50 IU/L and 38.00 IU/L respectively). The LDL levels were significantly influenced by the treatments. The control group (T₁) had the highest LDL value (24.50 mg/dl), which was significantly higher than the other treatment groups. The group with 50 ml/kg *J. carnea* + 25 ml/kg *O. gratissimum* (T₄) had the lowest LDL value (19.50 mg/dl) among all the treatment groups. The groups with 50 ml/kg *J. carnea* (T₂) and 25 ml/kg *O. gratissimum* (T₃) had statistically similar LDL values (21.50 mg/dl). For HDL, the group with 50 ml/kg *J. carnea* + 25 ml/kg *O. gratissimum* (T₄) had the highest HDL value (63.50 mg/dl), which was significantly higher than the other treatment groups. The control group (T₁) had the lowest HDL value (51.50 mg/dl), which was significantly lower than the other treatment groups. The group with 50 ml/kg *J. carnea* + 0 ml *O. gratissimum* (T₂) had an intermediate HDL value (60.50 mg/dl), which was significantly higher than the control group. In total cholesterol, 0 ml/kg *J. carnea* + 25 ml/kg *O. gratissimum* (T₃) and 50 ml/kg *J. carnea* + 25 ml/kg *O. gratissimum* (T₄) were significantly lower than the control (T₁). Similarly, in creatinine, the control (T₁) had the highest value which was significantly ($p < 0.05$) different from T₂, T₃ and T₄ that were themselves similar.

4. Discussion

4.1. Growth Performance

In this research, it was noted that fish fed with extract from the *J. carnea* (T₂) demonstrated superior performance compared to those fed with *O. gratissimum* methanolic leaf extract (T₃) showing the highest body weight gain, feed intake, and final body weight. This outcome is likely attributed to the nutritional components of *Justicia carnea* with superior protein and amino acid contribution when compared with *O. gratissimum*. It could also be as a result of higher mineral density and presences of antioxidants and some phytochemicals such as carotenoid, flavonoids, and alkaloids (Andrew *et al.*, 2024). It was also observed that the treatment groups out-performed the control group in all the performance indices evaluated.

The work of Marroh-Eseoghene *et al.* (2016) corresponds with the findings from this work. He reported that *Clarias gariepinus* fed with 10ml of *Ocimum gratissimum* exhibited higher weight gain, better feed conversion ratio and total fish length which is in tandem with the findings from this present work. It was also observed that both leaf extracts performed better than the control group which shows the nutritional adequacy of the two plant leaf extracts. Afe *et al.* (2019) also reported that *Ocimum gratissimum* improved the growth of *Heterobranchus bidorsalis* (*H. bidorsalis*). The effect of *Ocimum gratissimum* on *Clarias gariepinus* using the feed intake (FI) feed conversion ratio (FCR) and weight

gain WTG) as marker showed that the optimum inclusion level of *Ocimum gratissimum* at 25 ml/kg could be linked to increased acceptability, improved microbiota, enhanced digestion and superior nutrient utilization in *C. gariepinus*.

More so, the phytochemical indices of the plant extracts revealed that they contain anti nutritional factors which could potentially have adverse effects on the fish when consumed in large quantity. These properties might have slightly influenced the digestibility, palatability (taste), and even the odor of the supplemented feed at higher inclusion level as in (T₄) which had the combination of the two plant extracts.

4.2. Hematological Response

The introduction of scent leaf (*Occimum gratissimum*) leaf extract into the dietary feed of female African catfish (*Clarias gariepinus*) has been noted to exert a significant influence on their hematological parameters. The Packed cell volume (PCV), Red Blood Cell (RBC) count and hemoglobin concentration (Hb) in this present study showed marked improvement in treatment groups supplemented with *J. carnea* and *O. gratissimum* extract compared to the control group. This work is in consonance with the work of Melo *et al.*, 2019; Ribeiro *et al.*, 2022 who reported similar findings when broiler birds were given diets supplemented with scent leaf meal. This observation implies a potential role of the bioactive constituents present in *Justicia carnea* and *Occimum gratissimum* leaf extract, such as flavonoids and phenolic compounds, in bolstering erythropoiesis and augmenting the oxygen-carrying capacity (Fernandes *et al.*, 2021). The rise in RBC values as reported in this present study tallies with the work of Oliveira *et al.*, 2022 who reported similar increase in red blood cell in their earlier study. This phenomenon is likely attributable to the stimulatory effects of *J. carnea* and *O. gratissimum* leaf extract on the proliferation and maturation of red blood cells (Oliveira *et al.*, 2022).

4.3. Serum Biochemistry

In this study, dietary supplementation with *Justicia carnea* and *Ocimum gratissimum* leaf extracts modulated these parameters without exceeding normal ranges. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) remained within reference limits, indicating preserved hepatocyte integrity and absence of hepatic stress (Akinrotimi *et al.*, 2012; Abdel-Tawwab *et al.*, 2020). The hepatoprotective effects are likely mediated by the antioxidant and anti-inflammatory phytochemicals, including flavonoids and phenolic compounds, present in both extracts (Halliwell & Gutteridge, 2015; Hashemi & Davoodi, 2011). These findings align with reports of improved liver enzyme profiles in fish fed phytogenic-supplemented diets (Melo *et al.*, 2019; Abdel-Tawwab *et al.*, 2021; Onu *et al.*, 2021).

Phytogenic inclusion significantly improved serum lipid profiles. Low-density lipoprotein (LDL) was highest in the control and lowest in the combined *J. carnea* + *O. gratissimum* treatment, indicating a synergistic hypolipidemic effect. Conversely, high-density lipoprotein (HDL) was markedly elevated in the combined treatment, reflecting enhanced reverse cholesterol transport and improved lipid clearance (Montero *et al.*, 2008; Abdel-Tawwab *et al.*, 2021). Total cholesterol was significantly reduced in fish fed 25 ml/kg *O. gratissimum* and the combined diet, supporting the hypocholesterolemic potential of these extracts through inhibition of cholesterol biosynthesis and stimulation of lipid catabolism (Hashemi & Davoodi, 2011; Reverter *et al.*, 2014; Dawood *et al.*, 2022).

Renal function was maintained, as evidenced by significantly lower creatinine levels in all extract-supplemented groups compared to the control (Gabriel *et al.*, 2011; Jimoh *et al.*, 2019). Reduced creatinine indicates stable kidney function and metabolic homeostasis, further confirming the safety of these phytogenic additives.

5. Conclusion

Dietary supplementation with *Justicia carnea* and *Ocimum gratissimum* leaf extracts significantly improved growth performance and feed utilization in African catfish compared with the control diet. The enhanced weight gain and feed conversion observed across the treatment groups suggest improved palatability, digestion, and nutrient utilization attributable to the plants' bioactive constituents. These findings confirm the potential of phytogenic feed additives as sustainable alternatives to synthetic growth promoters and antibiotics in aquaculture.

Recommendations

Justicia carnea and *Ocimum gratissimum* leaf extracts are recommended as natural feed additives in African catfish production. The inclusion levels of *J. carnea* and *O. gratissimum* identified in this study should be adopted in feed formulation to enhance performance and reduce feed costs. Further research should evaluate long-term effects on immunity, health status, and economic returns under commercial farming conditions.

Compliance with ethical standards

All fish-related experimental procedures were performed in compliance with established ethical guidelines for animal research as approved by the University of Nigeria, Nsukka (UNN). The study protocol received formal approval from the Institutional Animal Ethics Committee of UNN (approval no. UNN/IAEC/2024/825). Throughout the study, fish were treated humanely in accordance with internationally recognized standards for the care and use of laboratory animals. Measures were implemented to reduce stress, pain, and discomfort during handling, feeding, and sampling. Prior to the start of the experiment, fish were adequately acclimatized, and all experimental activities were conducted by trained personnel to ensure high standards of animal welfare throughout the study period.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest to be disclosed.

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