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# Phytochemical profile and growth performance evaluation of African catfish (*Clarias gariepinus*) fed soursop (*Annona muricata*) leaf meal

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ABSTRACT

The potential of *Annona muricata* leaf meal (SLM) as a feed additive for African catfish (*Clarias gariepinus*) was investigated through a 56-day feeding trial. The study began with a quantitative phytochemical analysis of SLM, followed by Gas Chromatography-Mass Spectrometry (GC–MS) of methanolic leaf extracts. 180 juveniles, averaging 151.12 ± 8.54 g, were randomly assigned to four groups, each with 45 fish and three replicates in a Completely Randomized Design. The groups–Control, T1, T2, and T3 were fed diets containing 0 %, 2.5 %, 5 %, and 7.5 % SLM, respectively. Phytochemical analysis revealed substantial amounts of phenols, flavonoids, alkaloids, and saponins. GC–MS identified 13 distinct compounds, including fatty acids, fatty acid esters, ethers and aldehydes. Fish were weighed at the study's conclusion, and blood samples were collected for hematological and serum lipid profile analysis. Condition factors and survivability were similar across all groups ($p \geq 0.05$). However, final body weight (FW), weight gain, specific growth rate, total feed intake (TFI), and feed conversion ratio varied significantly ($p < 0.05$). The control group exhibited the highest FW (339.20 ± 3.16 g) and TFI (597.05 ± 6.65 g), with results comparable to T1. Conversely, TFI and growth progressively declined with increasing SLM levels, with T3 showing the lowest FW (299.62 ± 7.83 g) and TFI (541.06 ± 20.39 g). Hematological and serum profile indices were similar across groups ($p \geq 0.05$). These findings highlight the nutritional and ethnopharmacological relevance of *A. muricata* leaves. It was,

*Abbreviations:* SLM, Soursop (*Annona muricata*) leaf meal; GC-MS, Gas Chromatography-Mass Spectrometry; DCP, Dicalcium phosphate; NFE, Nitrogen-free extract.

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therefore, concluded that SLM can be included in catfish diets at 2.5 % without negatively affecting growth or health.

## Introduction

The shortage of animal protein presents a significant challenge to food security in many developing countries. As the global population continues to rise, factors such as economic growth, urbanization, technological advancements, and better nutritional awareness are expected to drive higher demand for quality animal products, including fish [1]. In response, many countries are intensifying efforts to develop their aquaculture sectors to enhance food security and promote sustainable food production [2].

Despite being the fastest-growing food-producing sector globally, aquaculture production still struggles to meet the rising demand for aquatic products. The growth rate is projected to decline from 4.6 % during 2007–2018 to 2.3 % between 2019 and 2030 [1,3]. This slowdown can be attributed to several factors, including environmental regulations, reduced availability of land and water resources, disease outbreaks due to intensified farming, and declining productivity gains [1,4].

The African catfish [*Clarias gariepinus* (Burchell, 1822)], a freshwater species native to Northern Africa, has become widespread across various regions of the continent. It is found nearly throughout Africa, with some exceptions in the Maghreb, Upper and most of Lower Guinea, and the Cape provinces (REF). Additionally, it has been introduced in Europe and several parts of Asia, such as Jordan, Israel, Lebanon, Syria, and southern Turkey [5]. The species is highly valued in tropical aquaculture due to its market appeal, omnivorous diet, rapid growth, hardiness, high fecundity, and productivity. Over the years, African catfish has emerged as a crucial commercial aquaculture species that significantly enhances food security and improves livelihoods in regions heavily reliant on fish as their primary protein source [6]. Consequently, the global growth of aquaculture has been matched by a significant rise in African catfish production, especially in Nigeria, where local outputs account for over 67 % of the worldwide total for this species [7,8].

Despite its economic relevance and potential, the aquaculture sector faces numerous challenges that hinder its contribution. A critical issue is the rising cost and limited availability of conventional aqua-feed and feed ingredients, particularly energy and protein sources like fish, soybean, and maize. This situation poses significant challenges to the profitability and sustainability of aquaculture production in Sub-Saharan Africa. The impact is especially severe in developing countries like Nigeria, Ghana, and Kenya, where food insecurity remains a persistent concern [6,9]. Furthermore, efforts to boost productivity through increased stocking densities in limited spaces have resulted in nutrient buildup and reduced water quality while increasing stress on fish populations and exacerbating the environmental impact of aquaculture [2]. The use of antibiotics and synthetic hormones to enhance fish health and growth has also raised concerns about drug-resistant genes and potential negative effects on human and environmental health [10,11]. Consequently, there is a growing interest in exploring safer, more affordable alternatives such as phyto-additives, nutraceuticals, prebiotics, and organic acids for sustainable aquaculture production.

Plants and their extracts are increasingly used to improve aquaculture growth, immune function, and disease control [12,13]. These plant materials are rich in natural compounds, including flavonoids, phenols, tannins, terpenoids, alkaloids, and steroids, which have probiotic-like effects. They can stimulate appetite, enhance digestive enzymes, promote gut health, improve nutrient transport efficiency, and support steroidogenic functions in fish [2,14–16]. One plant that has garnered significant attention is *Annona muricata* Linnaeus (1753), commonly known as soursop or graviola. Native to Central and South America but now widely distributed globally [17,18], various parts of this plant, including the fruit, seeds, leaves, stems, and roots, have been traditionally used in ethnomedicine across tropical and subtropical regions due to their health benefits and high antioxidant content [19–22].

Notably, *A. muricata* leaves have gained prominence for their therapeutic potential. Traditionally used to treat ailments ranging from cystitis and headaches to more serious conditions like cancer [18], these leaves are rich in bioactive compounds (primarily, annonaceous acetogenins among other phytochemicals) with anti-inflammatory, antimicrobial, antioxidant, and anticarcinogenic properties believed to contribute to their medicinal value [21,23,24]. Previous studies have linked the blood sugar- and fat-lowering effects of *A. muricata* leaves to their antioxidant activity [25–27]. However, despite these recognized health benefits, the specific bioactive compounds responsible remain largely unidentified, necessitating further chemical analysis. Gas chromatography-mass spectrometry (GC–MS) has emerged as a crucial technique for identifying and quantifying bioactive chemicals in plants, even at minute concentrations [28,29].

While most studies on the biological benefits of *A. muricata* leaves have focused on animal models such as laboratory rats and mice, its dietary inclusion has been reported to improve [30] or have no significant effect [31] on the growth performance of West African Dwarf goats. Ochokwu et al. [32] previously reported enhanced growth performance and gonadal development in the F1 generation fry of *C. gariepinus* broodstock fed diets containing up to 4 % *A. muricata* leaf meal. Additionally, Jiwuba et al. [33] documented improved hematological and serum biochemical indices in broiler chickens administered *A. muricata* leaf extracts. The antimicrobial and health-enhancing properties of these extracts have also been demonstrated in *C. gariepinus* and *Oreochromis niloticus* (Linnaeus, 1758) [13]. However, there is limited information on the effects of feeding this plant on the growth performance of *C. gariepinus* juveniles.

Therefore, this study aims to assess the phytochemical composition of *A. muricata* methanolic leaf extract using GC–MS profiling. Understanding this composition could enhance our knowledge of the plant's nutritional and therapeutic capabilities while offering new prospects for developing innovative natural products in pharmaceuticals and medicine. A subsequent feeding trial was conducted to evaluate the nutritional potential of this leaf by assessing the growth performance along with hematological and serum lipid profile indices in juvenile *C. gariepinus*.

## Methodology

### *Procurement and processing of soursop leaf*

Lush leaves of soursop / Graviola (*Annona muricata* L.), locally known as "shawashop", were procured from Igbo-Ukwu town in Anambra state, Nigeria, at the GPS coordinate of N6.01607°, E7.02103° The herbarium at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, provided its authentication (University of Nigeria Herbarium, UNH No 8a). The plant name was checked and confirmed on https://www.worldfloraonline.org on May 01, 2024. The leaves were firstly washed in plastic bowls with clean water, followed by draining in plastic sieves and subsequently air-drying at ambient conditions for seven days. Afterward, the dry leaves were pulverized into a fine powder (soursop leaf meal, SLM) and then stored for phytochemical analysis and fish diet formulations. The SLM was preserved in airtight, opaque, and moisture-resistant plastic containers and kept in a cool, dry environment at room temperature (20 - 25 °C) to minimize degradation of phytochemicals or possible mold growth.

### *Phytochemical evaluation of SLM*

#### *Chemicals and reagents*

All the chemicals and reagents used in the present study were of analytical grade and were procured from Guangdong Chemical Factory (Guangdong, China) and Sigma-Aldrich (USA).

#### *Proximate analysis*

For the Proximate composition, samples of the Soursop leaf meal (SLM) were analyzed for the proximate indices of crude protein (CP), crude fibre (CF), ether extract (EE), ash, moisture (Moist.), and nitrogen-free extract (NFE) following standard methods of the Association of Official Analytical Chemists [34].

#### *Quantitative phytochemical assessment*

Quantitative analysis of the SLM's phytochemical compositions was conducted to ascertain the component levels of flavonoids, alkaloids, phenol, saponins, and tannins.

*Determination of alkaloids:* The alkaloid determination followed the methods described by Obadoni and Ochuko [35]. Initially, 200 mL of a 20 % acetic acid solution was combined with 5 g of SLM in a covered 250 mL beaker and left standing for 4 h. The resulting solution underwent filtration and subsequent condensation to one-quarter of its initial volume using a water bath. Concentrated ammonium hydroxide was then added gradually till complete precipitation was achieved. After settling, the precipitate was filtered, collected, and weighed. The total alkaloid was determined as a percentage using the following formula (I):

$$Total\ alkaloids\ (\%) = Weight\ of\ residue(g) \times 100/Weight\ of\ original\ sample\ taken\ for\ analysis(g) \quad (I)$$

*Quantification of flavonoids:* The quantification of flavonoids followed the approach outlined by Harborne [36]. In summary, 5 g of leaves underwent a 30 min reflux boiling in 2 M HCl, followed by filtration after cooling. Subsequently, an equivalent volume of ethyl acetate was gradually added to the filtrate. The weight of the resulting precipitated flavonoids was measured and reported in percentage.

*Determination of saponin:* The quantification of saponin followed the procedure outlined by Obadoni and Ochuko [35]. Initially, 20 g of SLM was combined with 100 ml of 20 % aqueous ethanol and agitated for 30 min. Next, the mixture was subjected to heating in a water bath at 55 °C for 4 h. Following this, the mixture was filtered, and the residue was subjected to another extraction using 200 ml of 20 % aqueous ethanol. The resulting extracts were concentrated to approximately 40 ml over a water bath at 90 °C. The concentrate was then transferred to a 250 ml separatory funnel, where it underwent two extractions with 20 ml diethyl ether, with retention of the aqueous layer. Further steps involved adding 60 ml n-butanol to the retained solution, followed by two washes with 10 ml of 5 % aqueous sodium chloride. The resulting solution was heated in a water bath, followed by evaporation, and drying (to a steady weight) in an oven at 40 °C. The percentage saponin composition was derived with the formula (II).

$$Saponin\ (\%) = Final\ weight\ of\ sample\ (g)\ \times\ 100/Initial\ weight\ of\ extract\ (g) \quad (II)$$

*Measurement of total phenolics*: Total phenolics were quantified following procedures outlined by Obadoni & Ochuko [35]. Initially, 5 g of SLM was boiled in 50 mL of ether for 15 min. The resulting extract was combined with distilled water at a ratio of 1:2. Subsequently, 2 mL of ammonium hydroxide and 5 mL of pentanol were included in the mixture, which was afterward, incubated at room temperature for 30 min. Total phenols were determined spectrophotometrically (Jenway® Spectrophotometer- Model 7315 UV/Visible single beam) by measuring absorbance at 505 nm.

*Measurement of tannin:* Tannin levels were assessed using Pearson's method [37]. Initially, 5 g of SLM was dissolved in 50 ml of distilled water and left for 30 min. The mixture was then intermittently shaken and centrifuged at 5000 × *g* to obtain the tannin extract, diluted in 100 ml of distilled water. In separate 50-ml volumetric flasks, 5 ml of the diluted extract and 5 ml of standard tannic acid solution (0.1 g/L) were prepared. Following this, 1 ml of Folin–Denis' reagent was added along with 2.5 ml of saturated sodium carbonate solution to each flask, with volumes adjusted to 50 ml using distilled water. The mixtures were subjected to a 90-minute incubation at room temperature afterward [38]. Spectrophotometric analysis was conducted at 760 nm against a reagent blank to

determine tannin content and expressed as a percentage.

*Extraction of crude fat from A. muricata leaves*

The SLM was subjected to crude fat extraction using the Soxhlet extraction technique with methanol (≥99.9 % purity) as the solvent (750 mL). The extraction process occurred over 48 h at a temperature below the solvent's boiling point. Subsequently, the resulting extract underwent filtration using a 45 μm filter paper and, afterwards, heated to concentration, yielding a concentrated methanol extract. This extract was refrigerated (at 4 °C) until used for analysis, following protocols outlined by Hsouna et al. [39].

*Gas chromatography-mass spectrometry (GC–MS) analysis of phytochemical components*

The methanolic extract's phytochemical compositions were analyzed utilizing a GCMS-QP2010 Plus Shimadzu system from Japan. The GC–MS system was set under standardized experimental conditions as outlined: Ion-source temperature was sustained at 200 °C, Interface temperature at 250 °C, Pressure at 16.95 psi, and Injector was set to 1 μl in split mode with a split ratio of 1:20, alongside an injection temperature of 250 °C. The column temperature commenced at 70 °C for 5 min, incrementally rose to 280 °C at a rate of 10 °C/min, and remained unchanged for 5 min, resulting in a total elution duration of 21 min. The relative percentage levels of the constituent bio-compounds were assessed by comparing their mean peak area to the total peak areas. Data acquisition and system control were facilitated using MS Solution software offered by the supplier. Identification of bioactive constituents within the leaf extract was accomplished by referencing the National Institute Standard and Technology (NIST) libraries—additionally, a comparison of retention indices aided in confirming the constituents' identity. The results were tabulated based on the comparison with available compounds in the computer library (NIST) connected to the GC–MS instrument.

*Experimental diet compositions*

Four experimental diets were formulated as isonitrogenous (38 % CP) and isocaloric (2.7 MCal/kg) to ensure comparable protein and energy content across all treatments. The diets were formulated with graded levels of SLM at 0 %, 2.5 %, 5 %, and 7.5 %, respectively, and were assigned and fed to the respective treatment groups as follows:

- Control – 0 % SLM.
- Treatment 1 (T1) – 2.5 % SLM.
- Treatment 2 (T2) – 5 % SLM.
- Treatment 3 (T3) – 7.5 % SLM.

After the formulations, the various treatment diets were also taken to the Nutrition and Biochemistry laboratory of the Department of Animal Science for proximate analysis. They were analyzed using the procedure described for the proximate analysis of the SLM above. The percentage ingredients and proximate compositions of the experimental diets used for the study are presented in Table 1.

*Ethical approval*

All experimental procedures complied with the ethical guidelines outlined in the University of Nigeria Research Policy Document

**Table 1**
Percentage composition of experimental diets.

| Ingredients | Treatments (Percentage SLM) | | | |
|---|---|---|---|---|
| | Control (0 %) | T1 (2.5 %) | T2 (5 %) | T3 (7.5 %) |
| Yellow Maize (%) | 14.7 | 11 | 12 | 11.00 |
| Wheat offal | 10 | 11.4 | 9 | 8.5 |
| Soybean Meal (%) | 33 | 33 | 32 | 31 |
| Fish Meal (%) | 35 | 34.8 | 34.7 | 34.7 |
| SLM (%) | 0 | 2.5 | 5 | 7.5 |
| Palm Oil (%) | 6 | 6 | 6 | 6 |
| Lysine (%) | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine (%) | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix (%) | 0.5 | 0.25 | 0.25 | 0.25 |
| Toxin binder (%) | 0.1 | 0.25 | 0.25 | 0.25 |
| DCP | 0.2 | 0.2 | 0.2 | 0.2 |
| Total (%) | 100 | 100 | 100 | 100 |
| **Chemical composition** | | | | |
| Crude protein (%) | 38.04 | 37.74 | 37.5 | 38.06 |
| Crude fibre (%) | 4.5 | 4.5 | 5 | 4.5 |
| Ether extract (%) | 7.5 | 6.5 | 6.5 | 6.5 |
| Ash (%) | 6.75 | 5.7 | 6.75 | 6.25 |
| Moisture (%) | 8 | 8.05 | 7.25 | 8.25 |
| NFE (%) | 35.21 | 37.51 | 37 | 36.44 |

SLM: Soursop leaf meal; DCP: Dicalcium phosphate; NFE: Nitrogen-free extract.

[40] to ensure the well-being of animal subjects. Ethical approval for this study was granted by the Faculty of Biological Sciences Research Ethics Committee, University of Nigeria, Nsukka, under approval number UNN/FBS/25/SS.10892.

*Experimental design and management of fish*

Healthy one hundred and twenty (180) African catfish juveniles (*C. gariepinus)* with an average weight of 151.12 ± 8.54 g were procured from a reputable commercial aquaculture farm in Nsukka town of Enugu State, Nigeria, and used for the study. The fish were transported in polythene bags filled with oxygen to the University Fish farm, where they were transferred into a concrete fishpond. The fish were initially acclimatized for two weeks and fed a commercial fish diet. Before commencing the study, the feed was withdrawn from the fish for a 24-hour period to eliminate weight differences due to food residue in their gut and stimulate their appetite. The fish were randomly assigned to four treatment groups of Control/T0, T1, T2, and T3, respectively, in a Completely Randomized Design which received the respective diets containing 0 %, 2.5 %, 5 %, and 7.5 % of SLM as earlier described for 56 days. Each treatment was replicated thrice with 15 fish per treatment and kept in plastic bowls (100 L capacity). The initial body weight and standard and total length were also measured and recorded before introducing the experimental diet to the fish. The fish were fed 5 % of their body mass daily, divided into two equal portions (2.5 % each) provided twice daily, commencing at 08:00 a.m. and 03:00 pm., respectively. During each feeding session, which lasted between 30 and 45 min, the fish were fed ad libitum. After each session, any uneaten food was carefully siphoned out to prevent water contamination and ensure accurate determination of feed intake. During the study, water quality indices were monitored regularly, using appropriate equipment, and the water was regularly changed (twice weekly) to maintain good quality. Water quality parameters (Table 2) were monitored periodically using calibrated equipment: pH (HI-98,127, Hanna Instruments, USA), dissolved oxygen (HI9147, Hanna Instruments, USA), ammonia (WQT-154, GAOTek Inc., Canada), and temperature (271,407, HENDI B.V., Netherlands). All parameters remained within the tolerance range for C. gariepinus [41,42]. Anti-stress (glucose) was administered to the fish water immediately after procurement, followed by subsequent weighing exercises during the study.

*Growth performance*

To evaluate the growth performance, the fish first fasted for 24 h. Afterward, each replicate group's body weight and total length measurements were taken under anesthetics (0.1 g Tricaine mesylate water$^{-1}$). Measurements were made at the onset and the end of the study. The determination of growth performance and calculation of feed utilization were made using the following formulae:

$$Weight\ gain\ (g) = Final\ weight\ (g) - Initial\ weight\ (g) \quad \text{(III)}$$

$$Percentage\ weight\ gain\ (\%) = Weight\ gain\ (g) \times 100\ / Initial\ weight\ (g) \quad \text{(IVa)}$$

$$Specific\ growth\ rate\ (\%g\ /\ day) = (InFinal\ weight - InInitial\ weight) \times 100/time\ (duration\ of\ the\ experiment) \quad \text{(IVb)}$$

Where *In* represents the natural logarithm

$$Feed\ conversion\ rate\ (FCR) = Total\ feed\ Intake\ (g)/Weight\ gain\ (g) \quad \text{(V)}$$

Where Total feed intake represents the sum of the feed offered to the fish throughout the study.

$$Condition\ factor\ (K) = Weight\ of\ the\ fish \times 100/Fish\ Length(L)^3 \quad \text{(VI)}$$

$$Survivability\ (\%) = Number\ of\ fish\ at\ the\ end\ of\ the\ study \times 100/\ Number\ of\ fish\ at\ the\ beginning\ of\ the\ study \quad \text{(VII)}$$

*Haematology*

At the end of the eight weeks, the fish were anesthetized as described earlier, and blood samples were collected from the dorsal arteries by inserting a 2 ml syringe a few centimeters into the flesh along the lateral line. Two mL of blood were collected into sterilized ethylenediaminetetraacetic acid (EDTA) collection bottles, labeled correctly, held in a cool environment, and then used for hematological assessment within a few hours of collection. The determinations of total red blood cell count (RBC) and total white blood cell counts (WBCs) were done using the new Neubauer hemocytometer. Staining and enumerations for the red blood cells were carried out as described by [43]. The Haematocrit values and hemoglobin concentrations were determined using the microhaematocrit capillary

**Table 2**
Water quality indices of the different fish treatment groups fed varying levels of soursop leaf meal.

| Treatment | pH | DO (mg/L) | Ammonia level (mg/L) | Temperature (°C) |
|---|---|---|---|---|
| Control | 7.4 ± 0.1 | 6.4 ± 0.15 | 0.11 ± 0.01 | 26.7 ± 1.2 |
| T1 | 7.3 ± 0.0 | 6.2 ± 0.14 | 0.12 ± 0.01 | 27.8 ± 1.4 |
| T2 | 7.3 ± 0.1 | 6.4 ± 0.09 | 0.12 ± 0.0 | 27.7 ± 0.9 |
| T3 | 7.3 ± 0.0 | 6.4 ± 0.13 | 0.11 ± 0.01 | 27.3 ± 0.6 |

DO: Dissolved oxygen.

tube and cyanomethemoglobin methods, respectively, as earlier described by [44]. Other hematological indices of mean corpuscular hemoglobin (MCH) (eq. VIII), mean corpuscular volume (MCV) (eq. IX) and mean corpuscular hemoglobin concentration (MCHC) (eq. X) were calculated from the primary hematological indices using the formulas described by Ware [45].

$$MCV = PCV \times 10/RBC \quad \text{(VIII)}$$

$$MCH = HB \times 10/RBC \quad \text{(IX)}$$

$$MCHC = HB \times 100/PCV \quad \text{(X)}$$

The fish were not fed in the evening preceding the day of blood sampling. Blood sampling and collection from the fish were carried out in the early morning hours when the temperature was relatively cooler and more stable to minimize stress-related physiological variations.

*Serum lipid profile*

Blood samples were collected and transferred into EDTA-free bottles for the serum lipid profile analysis. After tilting the bottles to separate the serum, it was carefully extracted using a pipette and stored in capped test tubes. These serum samples were then transported to the laboratory and stored at −20 °C until used for biochemical analysis. A lipid-plus measuring system (Jant Pharmacal Corporation, Encino, CA.) was employed to determine the lipid profile, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglyceride levels (TAG).

*Statistical data analysis*

The results of the proximate and phytochemical compositions of the SLM were presented as the mean (± standard deviation). They were plotted in a bar chart using the ggplot2 package in R. Before analyses, the normal distribution and homogeneity of variances were tested with Shapiro-Wilk and Levene's tests, respectively. Growth performance, hematology, and serum lipid profile data were subjected to One-way Analysis of Variance (ANOVA) using R 3.5 [46], the Agricolae package [47] separated the significant differences between means using Tukey's Honestly Significant Difference (HSD) test function.

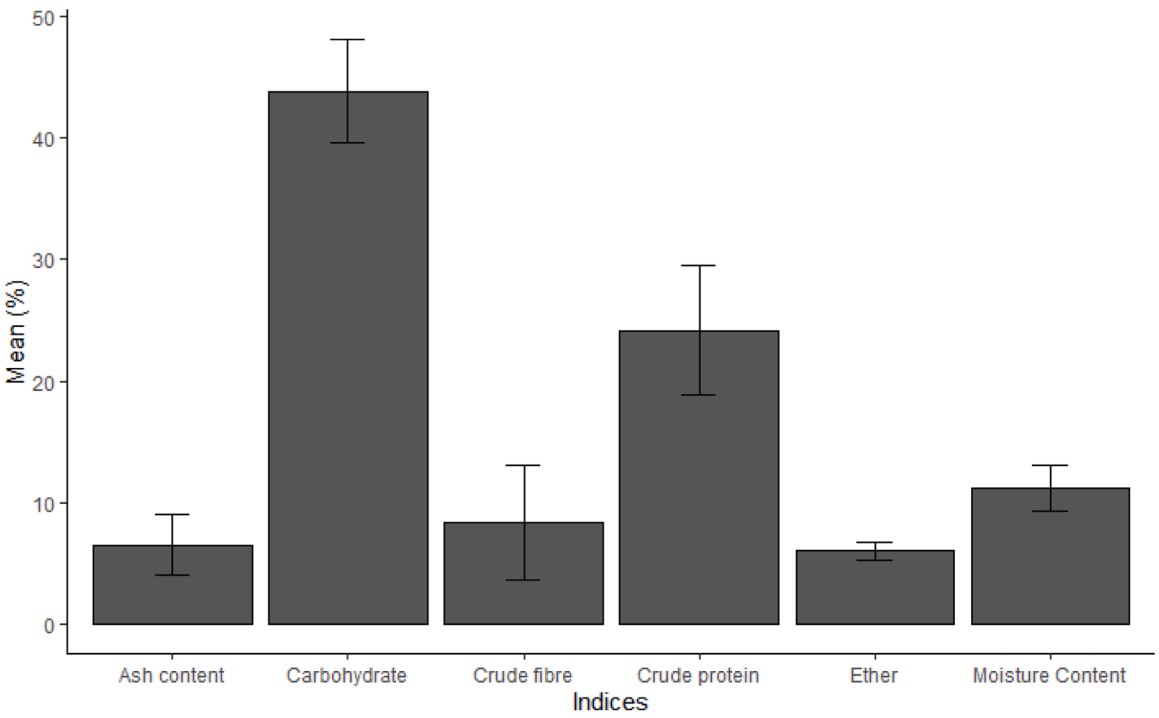


**Fig. 1.** Proximate Composition of *Annona muricata* leaf meal (Mean ± SD).

## Results

### *Phytochemical analysis of SLM*

#### *Proximate compositions*

The mean (± SD) of the proximate compositions of the SLM used in the present study are presented in Fig 1. The results show that SLM had high percentage levels of carbohydrate (43.82 ± 4.25 %) and CP (24.16 ± 5.28 %), with moderate amounts of Moist. (11.15 ± 1.91 %) and CF (8.33 ± 4.7 %). The leaf showed lower percentage levels of ash content (6.53 ± 2.5 %) and crude fat (6.02 ± 0.74 %) compared to other indices.

#### *Phytochemical compositions of annona muricata leaves*

Table 3 summarizes the findings of the phytochemical analysis conducted on the *Annona muricata* leaf extract (SLM). The phytochemical profiling showed various compounds, incorporating phenols, flavonoids, alkaloids, saponins, and tannins. SLM exhibited substantial amounts of phenols and flavonoids, constituting 9.5 ± 0.00 % and 8.6 ± 0.13 %, respectively. Moderate levels of alkaloids (5.75 ± 1.06 %) and saponins (5.05 ± 0.21 %) were observed, while tannin presence was minimal (0.074 ± 0.01 %).

#### *GC–MS analysis of annona muricata leaf extract*

Further GC-MS assessments revealed 13 distinct compounds in diverse chemical classes, including carboxylic acids, esters, ethers, and aldehydes in *A. muricata* leaf methanolic extract. These bioactive compounds, along with their common and IUPAC names, molecular formula, molecular weight, retention time, peak area, and classification of compounds, are detailed in Table 4. Additionally, the GC chromatogram (Fig. 2) illustrates the distribution of these compounds, highlighting the significant constituents of *A. muricata* leaves by their respective retention times.

The prominent compounds identified in *A. muricata* leaves with their retention times include (Z)-octadec-9-enoic (oleic) acid (17.787), Methyl 14-methylpentadecanoate (15.488), Methyl octadecenoate (17.429), Hexadecanoic (palmitic) acid (16.067), Methyl (E)-octadec-11-enoate (17.231), and Octadecanoic (stearic) acid (17.957). Notably, the fatty acid (Z)-Octadec-9-enoic (oleic) acid exhibited the highest peak area (27.53 %), whereas the aldehyde, (E)-2-Ethylhex-2-enal, recorded the least peak area (0.4 %).

### *Feeding trial*

#### *Growth performance of fish*

The results of the growth performance of African catfish fed varying SLM are presented in Table 5. Results showed no significant differences in the initial body weight ($p = 0.79$), condition factor ($p = 0.26$), and survivability ($p = 0.22$) of the fish. However, the final body weight (FW), weight gain (WG), percentage weight gain (PWG), specific growth rate (SGR), total feed intake (TFI), and feed conversion ratio (FCR) differed ($p < 0.05$) across the treatments. Fish in the control group had the highest ($p < 0.05$) FW (339.20 ± 3.16 g), WG (191.20 ± 6.35 g), PWG (129.81 ± 8.87 %), SGR (1.50 ± 0.07 % g/day), and TFI (597.05 ± 6.65 g), which were similar to those of T1. The growth performance indices were seen to decrease with further increase in the dietary level of SLM, with T3 showing the lowest values for FW (299.62 ± 7.83 g), WG (149.27 ± 2.16 g), PWG (99.52 ± 3.11 %), SGR (1.23 ± 0.03 %g/day), and TFI (541.06 ± 20.39 g). On the other hand, the FCR value was lowest in the control group (3.13 ± 0.14) and appeared to increase with increasing dietary SLM levels with T2 (5 % SLM) having the highest ($p < 0.05$) level of FCR (3.80 ± 0.15).

#### *Hematology*

Table 6 presents the effects of dietary levels of SLM on the hematological indices of *C. gariepinus* juveniles. Results showed that there were no significant differences among the treatment groups in all the hematological indices of red blood cell (RBC) ($p = 0.50$), white blood cell (WBC) ($p = 0.14$), packed cell volume (PCV) ($p = 0.69$), hemoglobin (HB) ($p = 0.70$), mean corpuscular volume (MCV) ($p = 0.25$), mean corpuscular hemoglobin (MCH) ($p = 0.74$), mean corpuscular hemoglobin concentration (MCHC) ($p = 0.74$), lymphocytes (LYM) ($p = 0.13$), monocytes (Mono) ($p = 0.59$), and granulocytes (Gran) ($p = 0.07$).

#### *Serum lipid profile*

Table 7 shows the results of the serum lipid profile of *C. gariepinus* juveniles fed varying levels of SLM. As observed, there were no significant differences in the TC ($p = 0.64$), TAG ($p = 0.80$), HDL ($p = 0.53$), and LDL ($p = 0.14$) levels of the fish across the groups.

**Table 3**
Results of the phytochemical analysis of Soursop (*Annona muricata*) leaf meal (Mean ± SD).

| Phytochemicals | Compositions (%) |
|---|---|
| Phenols | 9.5 ± 0.00 |
| Flavonoids | 8.6 ± 0.13 |
| Alkaloids | 5.75 ± 1.06 |
| Saponins | 5.05 ± 0.21 |
| Tannins | 0.74 ± 0.01 |

**Table 4**
GC–MS spectral analysis of *Annona muricata* methanolic leaf extract.

| S/N | Compound (Common names) | IUPAC nomenclature | Molecular formula | MW g/mol | RT (Min) | Peak Area % | Nature of Compound |
|---|---|---|---|---|---|---|---|
| 1 | 1,1-Dimethoxyhexane | 1,1-Dimethoxyhexane | $C_9H_20O_2$ | 146 | 3.8 | 0.49 | Ether |
| 2 | 2-Ethyl-2-Hexenal | (E)-2-Ethylhex-2-enal | $C_8H_{14}O$ | 126 | 4.119 | 0.4 | Aldehyde |
| 3 | Dodecanal Dimethyl Acetal | 1,1-Dimethoxydodecane | $C_{14}H_{30}O_2$ | 230 | 11.902 | 0.92 | Ether |
| 4 | Methyl 14-methylpentadecanoate | Methyl 14-methylpentadecanoate | $C_{17}H_{34}O_2$ | 270 | 15.488 | 15.2 | Fatty Acid Ester |
| 5 | Palmitic acid | Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 16.067 | 11.5 | Fatty Acid |
| 6 | 11-Octadecenoic acid, methyl ester | Methyl (E)-octadec-11-enoate | $C_{19}H_{36}O_2$ | 296 | 17.231 | 11.44 | Fatty Acid Ester |
| 7 | Methyl stearate | Methyl octadecanoate | $C_{19}H_{38}O_2$ | 298 | 17.429 | 12.75 | Fatty Acid Ester |
| 8 | Oleic Acid | (Z)-Octadec-9-enoic acid | $C_{18}H_{34}O_2$ | 282 | 17.787 | 27.53 | Fatty Acid |
| 9 | Stearic acid | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 17.957 | 8.72 | Fatty Acid |
| 10 | Methyl Linolenate | Methyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate | $C_{19}H_{32}O_2$ | 292 | 18.885 | 2.95 | Fatty Acid Ester |
| 11 | Glyceryl 2-Pentadecanoate | 1,3-Dihydroxypropan-2-yl pentadecanoate | $C_{18}H_{36}O_4$ | 316 | 18.96 | 1.16 | Fatty Acid Ester |
| 12 | Methyl (7E,10E)-Hexadeca-7,10-Dienoate | Methyl (7E,10E)-hexadeca-7,10-dienoate | $C_{17}H_{30}O_2$ | 266 | 20.262 | 4.75 | Fatty acid Ester |
| 13 | 9-Octadecenal | (E)-Octadec-9-enal | $C_{18}H_{34}O$ | 266 | 20.451 | 2.19 | Aldehyde |

*GC–MS: Gas Chromatography-Mass Spectrometry; IUPAC: International Union of Pure and Applied Chemistry; MW: Molecular Weight; RT: Retention Time.

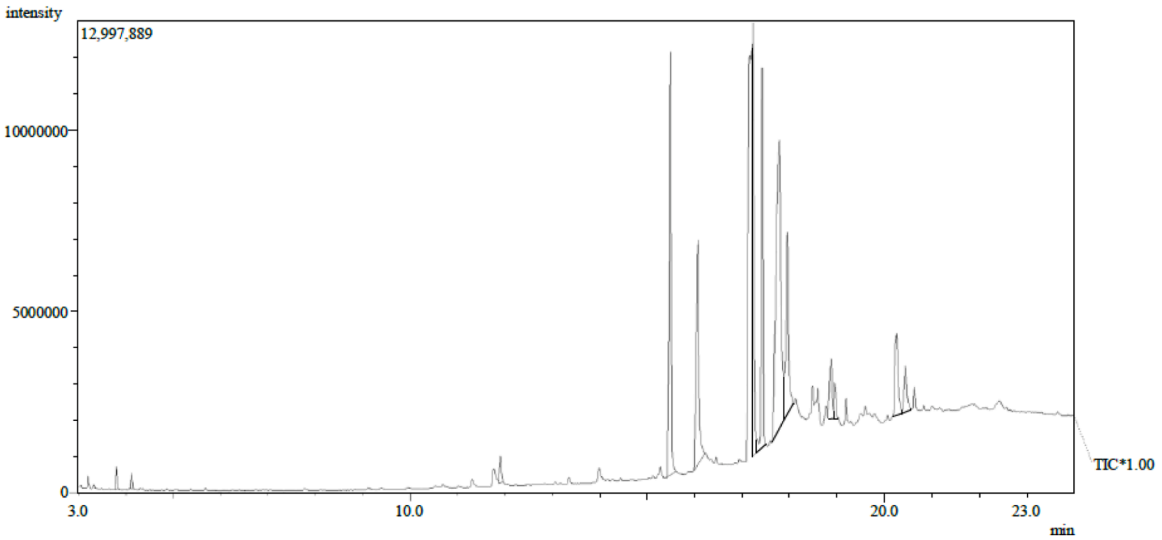


**Fig. 2.** GC–MS Chromatogram of *Annona muricata* methanolic leaf extract.

## Discussion

The escalating prevalence of drug-resistant microorganisms and the detection of drug and hormone residues in aquatic environments, fish, and humans call attention to the urgent need for sustainable and eco-friendly aquaculture practices. The potential health risks associated with these contaminants have spurred a renewed interest in natural alternatives. Historically, plants have been a cornerstone of medicinal therapies, and their bioactive compounds continue to inspire modern drug development [48]. Phytochemicals, especially those derived from medicinal herbs, offer a promising avenue for developing sustainable aquaculture strategies while mitigating the risks posed by synthetic products [49].

Soursop (*A. muricata*) is renowned for its rich phytochemical profile and traditional medicinal applications. A comprehensive understanding of its phytochemical composition is essential to harness its potential in animal nutrition, pharmaceutical, and industrial processes. This study aimed to accurately identify and quantify the phytochemical constituents of soursop leaves and evaluate their impact on the growth performance, hematology, and lipid profile of *C. gariepinus* catfish juveniles through a 56-day feeding trial.

The proximate analyses of feed materials are usually carried out to assess their nutritional quality. The high levels of CP and carbohydrate observed in SLM suggest its potential as an essential source of energy and protein in aquaculture and livestock feeds. Carbohydrates supply the energy needed for cellular metabolism and other biochemical processes like lipogenesis and biosynthesis of nucleotides and amino acids [50]. Dietary proteins serve as alternative energy sources and are primarily used for building and

**Table 5**
Growth performance of African catfish fed varying levels of Soursop leaf meal (SLM) Data expressed as mean ± standard deviation (SD) of three replicates.

| Parameters | Diet groups (g/100 g) | | | | |
|---|---|---|---|---|---|
| | 0 % SLM (Control) | 2.5 % SLM (T1) | 5 % SLM (T2) | 7.5 % SLM (T3) | P-Value |
| IW (g) | 147.98 ± 5.21 | 150.51 ± 6.07 | 155.6 5 ± 4.14 | 150.34 ± 6.07 | $0.79^{NS}$ |
| FW (g) | $339.20 \pm 3.16^{a}$ | $334.23 \pm 8.43^{ab}$ | $306.34 \pm 7.90^{bc}$ | $299.62 \pm 7.83^{c}$ | 0.009* |
| WG (g) | $191.20 \pm 6.35^{a}$ | $183.72 \pm 2.44^{a}$ | $150.70 \pm 5.96^{b}$ | $149.27 \pm 2.16^{b}$ | < 0.001* |
| PWG (%) | $129.81 \pm 8.87^{a}$ | $122.35 \pm 3.57^{ab}$ | $96.92 \pm 4.23^{bc}$ | $99.52 \pm 3.11^{c}$ | 0.006* |
| SGR (%g/day) | $1.50 \pm 0.07^{a}$ | $1.42 \pm 0.03^{ab}$ | $1.21 \pm 0.15^{c}$ | $1.23 \pm 0.03^{bc}$ | 0.005* |
| TFI (g) | $597.05 \pm 6.65^{a}$ | $606.13 \pm 10.21^{a}$ | $571.24 \pm 6.35^{ab}$ | $541.06 \pm 20.39^{b}$ | 0.022* |
| FCR | $3.13 \pm 0.14^{b}$ | $3.30 \pm 0.03^{ab}$ | $3.80 \pm 0.15^{a}$ | $3.62 \pm 0.12^{ab}$ | 0.016* |
| K | 1.00 ± 0.01 | 1.25 ± 0.2 | 1.07 ± 0.03 | 1.07 ± 0.07 | $0.26^{NS}$ |
| Survivability | 100 ± 0.0 | 93.3 ± 5.77 | 100 ± 0.0 | 96.7 ± 5.77 | $0.22^{NS}$ |

* : row means with different superscripts (a,b,c) are significantly different (Tukey's HSD test, $p < 0.05$)
NS: Non-Significant; IW: Initial Body Weight; FW: Final Body Weight; WG: Weight Gain; PWG: Percentage Weight Gain; TFI: Total Feed Intake; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; K: Condition factor.

**Table 6**
Hematology of African catfish juveniles fed varying levels of Soursop leaf meal (SLM) Data expressed as mean ± standard deviation (SD).

| Parameters | Treatments (Percentage SLM) | | | | |
|---|---|---|---|---|---|
| | 0 % SLM (Control) | 2.5 % SLM (T1) | 5 % SLM (T2) | 7.5 % SLM (T3) | P-Value |
| RBC ($\times 10^{12}$/l) | 1.91 ± 0.28 | 2.10 ± 0.15 | 2.20 ± 0.16 | 2.43 ± 0.29 | $0.50^{NS}$ |
| WBC ($\times 10^{9}$/l) | 55.99 ± 4.90 | 49.68 ± 1.35 | 53.12 ± 5.83 | 65.39 ± 5.03 | $0.14^{NS}$ |
| PCV (%) | 30.63 ± 2.75 | 30.61 ± 2.59 | 32.54 ± 3.02 | 34.30 ± 1.28 | $0.69^{NS}$ |
| HB (g/dl) | 9.70 ± 1.21 | 9.90 ± 0.86 | 10.38 ± 1.23 | 11.19 ± 0.19 | $0.70^{NS}$ |
| MCV (fL) | 170 ± 44.8 | 150 ± 45.1 | 149 ± 12.0 | 152 ± 54.4 | $0.25^{NS}$ |
| MCH (pg) | 51.9 ± 6.33 | 47.0 ± 3.21 | 47.3 ± 6.97 | 48.4 ± 11.8 | $0.74^{NS}$ |
| MCHC (g/dl) | 31.3 ± 3.79 | 33.6 ± 10.9 | 31.6 ± 2.58 | 32.9 ± 4.08 | $0.74^{NS}$ |
| LYM ($\times 10^{9}$/l) | 46.35 ± 4.96 | 34.41 ± 3.09 | 39.67 ± 6.33 | 50.22 ± 4.04 | $0.13^{NS}$ |
| Mono ($\times 10^{9}$/l) | 6.49 ± 0.67 | 9.08 ± 1.94 | 8.13 ± 1.96 | 6.80 ± 0.84 | $0.59^{NS}$ |
| Gran ($\times 10^{9}$/l) | 3.73 ± 0.43 | 6.24 ± 1.07 | 5.50 ± 0.60 | 4.45 ± 0.18 | $0.07^{NS}$ |

NS: Non-Significant; RBC: Red Blood Cell; WBC: White Blood Cells; PCV: Packed cell volume; HB: Haemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; LYM: Lymphocytes; Mono: Monocytes; GRAN: Granulocytes.

**Table 7**
Serum lipid profile of African catfish juveniles fed varying levels of Soursop leaf meal (SLM) Data expressed as mean ± standard deviation (SD).

| Parameters | Treatments (Percentage SLM) | | | | |
|---|---|---|---|---|---|
| | 0 % SLM (Control) | 2.5 % SLM (T1) | 5 %SLM (T2) | 7.5 % SLM (T3) | P-Value |
| TC (mmol/L) | 3.62 ± 0.24 | 4.26 ± 0.66 | 3.65 ± 0.30 | 4.31 ± 0.63 | $0.64^{NS}$ |
| TAG (mmol/L) | 1.92 ± 0.21 | 1.98 ± 0.11 | 1.79 ± 0.16 | 1.78 ± 0.20 | $0.80^{NS}$ |
| HDL (mmol/L) | 1.77 ± 0.12 | 2.1 ± 0.37 | 1.91 ± 0.08 | 1.68 ± 0.10 | $0.53^{NS}$ |
| LDL (mmol/L) | 2.01 ± 0.24 | 2.44 ± 0.35 | 1.69 ± 0.04 | 1.75 ± 0.24 | $0.14^{NS}$ |

Non-Significant ($p \geq 0.05$); Chol: Cholesterol; TAG: Triglyceride; HDL: High-density lipoproteins; LDL: Low-density lipoproteins.

repairing body tissues, as well as for the synthesis of hormones, enzymes, antibodies, DNAs, and other biological materials necessary for the maintenance of the animal's physiology and health [51]. The lower percentage Moist. content of SLM indicates its potential for long-term storage without the risk of microbial contamination and nutritional deterioration. The considerable levels of dietary fibre in SLM reflect its potential to enhance gut biology and functionality in animals by stimulating digestive tract function and enhancing food movement, digestion, and absorption [52]. The lower percentage levels of ash and ether in the SLM show a relatively lower composition of minerals and crude fat, respectively, in the SLM. Dietary fat is crucial for increasing feed flavor and palatability, insulating the animal's body, absorbing fat-soluble vitamins, and supporting optimal growth, reproduction, and immune system function [53]. Our results align with previous reports [54] of higher CP and lower Moist. levels in *A. muricata* leaves, as well as higher carbohydrate and lower CF and ash levels [50,55]. However, some disparities were noted in CP and fat levels compared to other studies [50,54–56]. These variations may be attributed to differences in plant variety, age/stage of harvest, climate, soil properties, and other growth and processing conditions, factors known to impact the nutritional and biochemical contents of plants [57,58]. Overall, our findings indicate that SLM is considerably higher in organic than inorganic compositions, highlighting its potential as a valuable feed ingredient in aquaculture. The comprehensive analysis of SLM's nutritional profile provides a foundation for its strategic incorporation into fish diets, potentially offering a sustainable and cost-effective alternative in aquaculture feed formulations.

As shown (Table 3), examining the constituent phytochemicals in *A. muricata* methanolic leaf extract uncovered several compounds known for antimicrobial, antioxidant, and pharmacological activities. Flavonoids and phenols act as free radical scavengers, exhibiting diverse functions such as anticancer, antibiotic, anti-inflammatory, cardioprotective, and immune system-boosting capabilities [59,60]. Alkaloids, offering predator and parasite-repelling traits to plants, also possess pharmacological properties, including antimicrobial, antihypertensive, antimalarial, and anticarcinogenic potentials [61]. Saponins, recognized for their bitter taste, exhibit growth stimulation, anti-inflammatory, antioxidative, hemolytic, and cholesterol-binding activities [62,63], in addition to serving as preservatives and flavorings [64,65]. Tannins, known for various properties including diuretic, antibacterial, antiviral, anti-inflammatory, anticarcinogenic, and antioxidant functions, also possess anti-nutritional and cytotoxic properties when consumed in excessive amounts [59,66]. Prior investigations highlighted that leaves exhibit the highest concentrations of phytochemicals among different plant parts [67]. This study adds further evidence to the rich phytochemical makeup of *A. muricata* leaves and their potential in treating and preventing infections. Similar phytochemical profiles and concentrations in *A. muricata* leaves were previously reported [54,68], while other research has shown lower percentage levels of these compounds compared to those observed in this study [50].

The GC–MS assessment of *A. muricata* leaf extract reveals a diverse array of bioactive substances with varying retention times and peak areas, highlighting its potential for industrial applications. Among the identified phytoconstituents (Table 4), fatty acids predominate, with oleic acid (OA) showing the highest peak area of 27.53 %, followed by palmitic acid (PA) at 11.5 % and stearic acid (SA) at 8.72 %. OA, a monounsaturated omega-9 fatty acid abundant in nature, plays a crucial role in cellular energy production and metabolic processes [69]. Studies associate increased OA consumption with preventive effects against diabetes mellitus, improved lipid profiles, and reduced risks of hypertension, heart disease, and cancer [69–71]. Additionally, OA exhibits anti-inflammatory and antioxidant properties [72,73].

PA and SA, saturated fatty acids found in animal and vegetable fats and endogenously synthesized, have garnered considerable research attention. PA demonstrates antioxidant properties, contributes to cellular membranes and secretory/transport lipids, and facilitates protein palmitoylation, activating signaling molecules [72,74,75]. However, excessive PA consumption is associated with overweight, type-2 diabetes, heart ailments, lipotoxicity, and cancerous growths [76–79]. SA exhibits antioxidative, anticonvulsant, and anti-carcinogenic activities, holding potential for drug delivery [80]. These fatty acids find applications in food processing, pharmaceuticals, cosmetics, and plastic/polymer industries [80–82]. The GC–MS analysis also confirmed the presence of various esters, including saturated (methyl 14-methylpentadecanoate and methyl stearate) and unsaturated (11-octadecenoic acid methyl ester, methyl linolenate, and methyl (7E,10E)-H\hexadeca-7,10-dienoate) fatty acid methyl esters (FAMEs), as well as the monoacylglyceride, glyceryl 2-pentadecanoate. Fatty acid esters, derived from the dehydration of fatty acids and alkyl alcohol, are highly valued for their diverse properties, including antimicrobial, anti-inflammatory, and antioxidant effects, along with physicochemical traits like moisturizing, conditioning, emulsifying, lubricating, and stabilizing actions [82,83]. Their utilization spans various sectors, including food, traditional medicine/pharmaceuticals, cosmetics, and biofuels [84–86].

Minute amounts of ethers (1,1-dimethoxyhexane and dodecanal dimethyl acetal) and aldehydes (2-ethyl-2-hexenal and 9-octadecenal) were also identified in the SLM leaf extract. Ethers find wide applications in industries ranging from rubber and plastics to medicine and cosmetics, serving various roles from refrigerants to anesthetics and chemical intermediates [87,88]. Aldehydes, including 2-ethyl-2-hexenal, are naturally reactive and toxic chemicals displaying pharmacological activities like antimicrobial effects and immunomodulation [89], with substantial applications in cosmetic, agrochemical, petrochemical, and polymer industries [90–92].

Our findings align with previous studies, such as Olasehinde et al. [27], who identified 22 chemical compounds in the methanolic leaf extract of *A. muricata* using GC–MS, encompassing various fatty acids, flavonoids, esters, phenols, aldehydes, and ketones. However, Souza et al. [93] reported a wide range of phytochemicals, including flavonoids and phenolics, in hydroalcoholic *A. muricata* leaf extract using HPLC methodology. This investigation provides valuable insights into the unique phytochemical compounds within the *A. muricata* leaf extract, highlighting its bioactivity, potential health benefits, and industrial applications. It reinforces the phytochemical, nutritional, and antioxidant potential of *A. muricata* leaves in ethno-medicinal practices [17], while also suggesting promising avenues for its use in aquaculture and animal nutrition.

Following the preliminary phytochemical assessment of SLM, a 56-day study was conducted to evaluate the effect of varying dietary levels of SLM on the growth performance, hematology, and lipid profile of *C. gariepinus* catfish juveniles. Analysis of variance revealed that dietary SLM inclusion above 2.5 % (at 5 % and 7.5 %) decreased ($p < 0.05$) the fish's FW, WG, PWG, and SGR. This reduced growth performance may be attributed to lower feed consumption observed in these groups. Annonaceae plants, including soursop, contain high levels of acetogenins, which inhibit the complex 1 respiratory chain (NADH ubiquinone reductase) and can suppress an animal's general metabolism, lowering feeding activity [94]. Additionally, SLM contains the alkaloid compound Annonain, which in excess can become bitter and toxic [95]. The high levels of SLM in T2 and T3 may have increased feed bitterness, lowering its palatability and acceptance by the fish. Similar reductions in feed intake have been reported in goats fed SLM-supplemented rations (up to 20 %) [31]. Tugiyanti et al. [96] also noted that SLM's saponin content can inhibit protease enzyme activity, impairing feed digestion and absorption. This may explain the higher FCR, and reduced growth observed in groups fed 5 % and 7.5 % SLM diets. Optimal fish growth requires that nutrient absorption exceeds basal metabolic demands. However, as Richard et al. [97] observed, alkaloids can be beneficial at low concentrations but toxic at higher levels. Nursal [98] further suggested that alkaloids can redirect energy from growth to detoxification under conditions of reduced feed intake, potentially explaining the lower SGR, PWG, and FW in fish fed higher SLM levels. The higher FCR values in SLM-treated groups compared to the control indicate impaired feed utilisation, with the most significant ($p < 0.05$) effect observed at 5 % SLM. These findings suggest that SLM inclusion above 2.5 % is detrimental to the growth performance and feed efficiency of African catfish. This aligns with Tugiyanti et al. [96], who reported reduced body weight gain in Tegal ducks fed SLM at 5 % or higher for five weeks. Similarly, Arthur et al. [99] found that high

doses (≥ 1000 mg/kg BW) of aqueous *A. muricata* extract caused appetite loss and decreased body weight in rats. In contrast, some studies have reported positive effects of SLM supplementation. Ochokwu et al. [32] observed improved growth and reproductive development in the F1 generation of *C. gariepinus* broodstock fed up to 4 % SLM. Maesaroh et al. [100] noted progressive improvements in FW, WG, and FCR in broilers administered soursop leaf extract or nano-encapsulated soursop leaf extract. Paul et al. [101] also reported enhanced WG in *Clarias batrachus* treated with *Annona squamosa* leaf extract, even after bacterial exposure. Additionally, Ali et al. [102] found no significant differences in growth parameters in mangrove crabs (*Scylla olivacea*) administered up to 600 ppm of *A. muricata* leaf extract, while Ndomou et al. [103] observed no significant effects on broiler growth when SLM was fed at 0.5 % for 49 days. These conflicting results may stem from differences in species, administration methods, concentrations, and durations of SLM supplementation. Such variability emphasises the importance of species-specific research and careful consideration of form, dosage and administration when incorporating SLM into animal diets.

Hematological parameters are valuable indicators of early changes in fish health status and can provide insights into phylogenetic relationships, physiological conditions, feeding habits, and immediate habitat conditions [104,105]. In our study, the observed hematological values fell within the normal physiological ranges for healthy juvenile C. gariepinus catfish, as reported by Adeyemo et al. [106] and Erhunmwunse & Ainerua [107]. The non-significant difference in the WBC count and LYM emphasizes the potential role of SLM in maintaining immunity and the general well-being of the fish [108]. Additionally, the consistent hemoglobin concentrations across all SLM inclusion levels suggest that dietary SLM did not induce any form of anemia. These findings are particularly noteworthy because, although the growth of the fish was hampered at higher levels of SLM inclusion, the results indicate that the fish's general health and physiology were not compromised. This observation is further supported by the similarity in the condition factor of the fish across treatments. Our results align with the reports of Jiwuba et al. [33], who observed no significant effect of soursop leaf extract (20, 40, and 60 mL/L) on the hematological indices of white blood cells, hemoglobin, and red blood cells of broiler birds, although they noted that packed cell volume was affected. Similarly, Arthur et al. [99] observed no significant differences in most hematological indices of male and female rats administered with up to 2500 mg/kg body weight of aqueous extract of *Annona muricata*, except for percentage lymphocytes. However, our findings contrast with those of Shukry et al. [109], who reported an increase in the hematological indices of Hb, RBC, WBC, and PCV in rats treated with *Annona muricata* extracts. These discrepancies highlight the potential for species-specific responses to SLM supplementation and underscore the importance of targeted research in different animal models. The findings of this study, therefore, indicate that the normal physiological function and health of the fish were not compromised by SLM inclusion. Thus, dietary levels of SLM up to 7 % inclusion in the diet of African catfish could be considered non-toxic to C. gariepinus catfish, despite the observed growth impairments at higher inclusion levels.

The non-significant effect of the dietary treatments on the lipid profile indices indicates that SLM had no impact on the lipid metabolism of C. gariepinus juveniles. Although previous studies have highlighted the hypolipidemic effects of *A. muricata* and its saponin content, which can limit cholesterol absorption and consequently reduce growth rates [110], our results did not suggest any interference with lipid metabolism in African catfish, even at higher SLM inclusion levels (≥ 5 %). This finding implies that the growth-limiting effects of SLM in fish may operate through alternative mechanisms rather than directly influencing lipid metabolism. These results are consistent with the findings of Arthur et al. [99], who demonstrated that administering aqueous extracts of *A. muricata* (up to 2500 mg/kg body weight) for 14 days had no significant effect on the lipid profile of male rats. Similarly, dietary inclusion of *A. muricata* flower meal at 5 g/kg showed no significant impact on TC, triglycerides (TAG), or LDL levels in broiler chickens. On the other hand, some studies have reported contrasting results. For instance, Shukry et al. [109] found that *A. muricata* extracts reduced TC, TAG, and LDL levels while improving HDL levels in rats at a dose of 200 mg/kg. Additionally, Alkassar and Alaboudy [111] reported that dietary inclusion of up to 0.4 % SLM progressively decreased serum cholesterol and TAG levels in broiler chickens. Conclusively, while *A. muricata* has demonstrated hypolipidemic effects in certain animal models, its lack of influence on lipid metabolism in C. gariepinus suggests species-specific metabolic responses or differing mechanisms underlying its observed growth-limiting effects. These findings emphasise the importance of exploring species-specific physiological responses when evaluating plant-based feed additives for aquaculture applications.

Generally, in the present study we report for the first time, the evaluation of SLM as a potential plant additive in African catfish production. The study marks a significant advancement in sustainable aquaculture practices. Our comprehensive approach, combining phytochemical assessment, GC–MS profiling, and feeding trials, provides novel insights into the complete potential of this natural feed additive. While we observed growth limitations at higher inclusion levels, the extract showed promising effects on fish health parameters. These findings contribute valuable data to the growing field of plant-based additives in aquaculture, addressing critical issues such as antibiotic resistance and environmental sustainability.

However, our study was limited by its focus on juvenile catfish and relatively short duration. Future research should explore long-term effects, optimal dosage for different life stages, and potential synergistic effects with other natural additives. Recent studies, such as Ali et al. [102] on mangrove crabs and Maryani et al. [112] on tilapia, suggest species-specific responses to *A. muricata* extracts, highlighting the need for broader investigations across diverse aquaculture species. Additionally, exploring nano-encapsulation techniques, as demonstrated by Maesaroh et al. [100], could enhance the efficacy and application of soursop leaf extract in aquaculture feeds. Future research could also focus on optimising SLM inclusion levels to balance potential health benefits with growth performance in various aquaculture species.

## Conclusion

In summary, this study unveiled the substantial phytochemical richness of *Annona muricata* leaves, comprising a diverse array of phenols, flavonoids, and other bioactive compounds with potential applications in medicine, animal agriculture, and industry. The

nutritional analysis of *A. muricata* leaf meal (SLM) revealed promising levels of carbohydrates, proteins, and lipids, supporting its traditional use in various ailments. To the best of our knowledge, this investigation represents the first exploration of SLM's impact on the growth performance, hematology, and lipid profile of *C. gariepinus* juveniles. Results indicate that incorporating SLM at 2.5 % into the diet did not adversely affect fish growth, suggesting its potential as a feed additive—however, higher inclusion levels (5 and 7.5 %) reduced feed intake and growth. While hematological and serum lipid parameters remained unchanged and within normal ranges during the study duration, further research is warranted to elucidate the biological effects of SLM at cellular levels. Also, given the plant's high antioxidant capacity, future studies should investigate its influence on cellular antioxidant defense mechanisms, endocrine function, reproductive biology, and potential toxicity in aquatic organisms. A comprehensive understanding of these aspects will be crucial for optimizing the utilization of *A. muricata* leaf meal in aquaculture and exploring its broader applications.

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## Declaration of generative artificial intelligence (AI) and AI-Assisted technologies in the writing process

The author(s) utilized ChatGPT to enhance the manuscript's readability and correct grammar during its preparation. After employing this tool/service, the author(s) critically reviewed and amended the content as necessary, assuming complete responsibility for the publication's accuracy and integrity.

## CRediT authorship contribution statement

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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