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EFFECTS OF STONE BREAKER *Phyllanthus niruri* LEAVES MEAL ON GROWTH PERFORMANCE AND IMMUNE RESPONSE IN *Clarias gariepinus* CHALLENGED WITH *Pseudomonas aeruginosa*

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Abstract

Effects of dietary supplementation of different inclusion levels of *Phyllanthus niruri* on *Clarias gariepinus* fingerlings induced with *Pseudomonas aeruginosa* was evaluated for 4-week. Two hundred and twenty-five (225) *C. gariepinus* fingerlings with a mean weight of 6.32±0.00g were randomly selected and allocated to five treatments of three replicates and each replicate comprises 15 *C. gariepinus* fingerlings. The control diet (negative) was without Stone Breaker Leaves (SBL) and the positive control was supplemented with 30 mg/kg Erythromycin. Other diets were supplemented with 1% and 2% of SBL and the combination of SBL and Erythromycin (SBL+ERY) (2%). Growth performance and nutrient utilization were calculated. Haematological and blood biochemical parameters were determined using standard methods. *Clarias gariepinus* fingerlings were inoculated with *P. aeruginosa* at 5.94 $\log_{10}$ CFU/ml intraperitoneally and fed with different diets to assess the percentage mortality and their Relative Percentage Survival (RPS). Fish fed treated diets had a higher growth rate than the control and there was significantly different ($P < 0.05$) among the dietary groups. The values of haematological and blood biochemical were better in the treated groups compared to that in the control group. However, the challenge test revealed that the treated groups recorded lower percentage mortality and a higher relative percentage of survival compared to the control group. The results indicated that supplementation of SBL and their combination with erythromycin (30mg/kg diet) could be used in fish farming to enhance growth performances. It also confirmed that *P. niruri* is capable of improving the immune response of *C. gariepinus*.

Keyword: *Pseudomonas aeruginosa,* Growth, *Clarias gariepinus, Phyllantus niruri,* Blood biochemical, Haematological parameter

Introduction

Fish is one of the main food components of human for many centuries and it constitutes an important part of the diet of many countries (Taiwo *et al.*, 2017). The disease causes serious economic losses and remains a major factor in the successful development of fish production. Drugs and synthetic antibiotics are used in animal feeds to promote feed efficiency, product quality, and control diseases. There is a problem of residual effects of drugs on animal products, the high cost of the drugs and availability (Omidiwura *et al.*, 2017). Natural plant

antimicrobials have made a significant impact on the prevention, control and treatment of infectious diseases in animals. Plant and plant extracts from herbs and spices are known to have medicinal properties (antimicrobial) can be used as an alternative to control disease and improved efficiency because they possess various bioactive compounds (Omidiwura *et al.*, 2017).

Phyllanthus niruri is an erect annual plant growing up to 40 - 70cm height having to ascend herbaceous branching; it is quite glabrous and branching at the base. The leaves are numerous, small, green, subsessile, closely arranged, elliptic oblong-shaped, obtuse, having short petiole and stipules present, they have arranged alternatively on each side of the stem (Bagalkotkar *et al.*, 2006). The flowers are yellowish, small, numerous, axillary. These are unisexual, monoecious flowers, male flowers having 1-3 sessile stamens and female flowers were solitary. The fruit is a capsule, very small, depressed globose and the capsule is smooth, 2-3mm in diameter, it is having horizontal branches and height of 30-60cm, 1-2.5mm width stem and the root. It is somewhat branched and large (Bagalkotkar *et al.*, 2006).

Different parts of *P. niruri* were reported to have various therapeutic activities such as expectorant, diaphoretic, carminative, laxative, astringent to the bowels, tonic to the liver, diuretic, earache, griping, ophthalmia and ascites, stomachic, aperitive, antispasmodic, diuretic, against constipation, fever including malaria, dysentery, gonorrhoea, syphilis, tuberculosis, cough, diarrhoea and vaginitis (Paranjape, 2001, Shanbhag *et al.,* 2010). With the increasing awareness of the potential of natural products using medicinal plants in the treatment of fish diseases, there is little or no information on the utilization of *P. niruri* in *C. gariepinus* nutrition. Hence, this study investigates the growth performance and immune response in *C. gariepinus* challenged with *P. aeruginosa.*

Materials and Methods

Study Area and Collection of *Phyllanthus niruri* Leaves

The study was conducted at the Fisheries and Aquaculture Technology unit of Teaching and Research Farm, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria. Fresh *P. ninuri* leaves were collected within the University premises and were identified and authenticated at the Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa.

Preparation of *P. niruri* Leaves and Formulation of Experimental Diets

The leaves were washed with sterile distilled water and air-dried for three weeks. The leaves were ground into a fine powder using a hammer mill. The leaves were added at 1% and 2% with other ingredients such as fishmeal, soybean, yellow maize, millet, starch, vegetable oil, dicalcium phosphate, vitamin-mineral premix and Erythromycin (30mg/kg diet) (Table 1). The ingredients were mixed manually and pelleted using a fabricated milling machine using 2mm diameter die. The resultant strands obtained were–sun-dried for three days and kept in an airtight–polyethylene bags and marked according to treatments and stored at room temperature to prevent mycotoxin formation until feeding.

Experimental Fish, Management and Feeding

Fifteen (15) *C. gariepinus* with an average weight of 6.32±0.00g were used for this study in 15 experimental bowls. Fish were subjected to a preventive bath of formaldehyde (37%, 30 min) and a quarantined was induced for four weeks before the commencement of the feedings trials without showing any disease

(no bruises and sign or symptoms of diseases was observed on the fish body) and the fish were fed commercial diets (2 mm Coppens of 40% crude protein) during this period. The feeding was stopped 24 h before the inception of the study. The fish were weighed using a sensitive weigh balance and divided into five treatments and three replicates in a complete randomized design. The fish were stocked in an experimental bowl (60 x 60 x 45 cm) of 45 L water capacity. All fish were fed at 3% body weight and feeding was done twice daily, 09:00 h and 17:00 h and the left-over feeds and faeces in each bowl were siphoned one hour after feeding. Water in each bowl was completely changed every three days and weight changes were determined weekly and feeding was adjusted according to the new bodyweight.

Table 1: Gross composition of the experimental diets (g/100g) fed to *C. gariepinus* fingerlings

INGREDIENTS	Control (0%)	SBL_2 (1%)	SBL_3 (2%)	ERY_4 (30mg/kg)	$(SBL+ERY)_5$ (2%)
Fish meal	16.79	16.79	16.79	16.79	16.79
Soybean	42.60	42.60	42.60	42.60	42.60
Yellow maize	16.31	16.31	16.31	16.31	16.31
Millet	16.31	15.31	14.31	14.31	14.31
Starch	1.00	1.00	1.00	1.00	1.00
Vegetable oil	2.00	2.00	2.00	2.00	2.00
DCP	2.00	2.00	2.00	2.00	2.00
*Vit-min premix	3.00	2.00	1.00	3.00	-
Stone breaker leaves	-	1.00	2.00	-	1.50
Erythromycin 30mg/kg	-	-	-	2.00	1.50
TOTAL	100.00	100.00	100.00	100.00	100.00
**Proximate composition of diets					
Moisture	9.46	10.20	10.30	9.49	9.44
Crude protein	44.70	45.40	45.44	45.40	45.20
Ether extract	3.76	4.13	4.13	3.65	3.81
Ash	10.80	11.20	11.20	11.30	11.80
Crude fibre	4.65	4.74	4.39	4.55	4.52
NFE	26.69	24.29	24.52	25.66	25.26

Note: SBL for Stone breaker leaves and ERY for Erythromycin, *vitamin - minerals premix. Each 2.kg of premix contain; 12.5 million international unit (MIU); D_3, 2.5 MIU;E, 40g; K_3 2g; B1,5.5g;BB6,5g; Niacin 55g; Calcium Pantothenate 11.5g; Chlorine chloride 500g; Folic acid, Biotin,0.08g;Manganese, 120g; Iron, 100g; Zinc, 80g, Copper,8.5g; Iodine, 1.5g;Cobalt,0.3g;Selenium, 0.12g; Anti-oxidant, 120g.** Proximate composition of experimental diet using standard methods of AOAC, (2005).

Test Organisms

Pseudomonas aeruginosa was isolated from *C. gariepinus and Oreochromis niloticus* juveniles. The isolation characteristics of bacteria using biochemical test were carried out at Microbiology Laboratory of Department of Biological Sciences, Olusegun Agagu University of Science and Technology, Okitipupa and the observation of characteristics were compared with characteristics in Bergey's manual. The pure cultures were sub-cultured on nutrient agar

and preserved in the refrigerator at 4°C until required for the study.

Challenge Test

Two hundred and twenty-five *C. gariepinus* (15 from each treatment) were challenged intraperitoneal with $5.94\log_{10}$cfu/ml of *P. aeruginosa* that was isolated from *C. gariepinus and O. niloticus.* The challenged fish were observed for clinical signs and mortality for four weeks. The mortalities were recorded and the relative percentage of survival (RPS) among the challenged fish was determined as described by Ibrahem *et al.,* (2010) and Olusola and Nwokike, (2018).

$$RPS = 1 - \frac{\text{(percentage of mortality in treated group) X 100}}{\text{(Percentage of mortality in the control group)}}$$

Growth performance

Growth performance was calculated after the experimental period as described by Olusola and Olawoye (2019).

Collection of Blood Samples

Blood samples (5 ml) were collected from *C. gariepinus* fingerlings at the beginning and end of 4 weeks feeding trials for haematological and blood biochemical. Three (3) *C. gariepinus* fingerlings were randomly selected from each replicate, the blood sample (2.5 ml) were collected from the fish using different 2 ml disposable heparinized syringes, with ethylene diamine tetra acetic acid (10ml EDTA) as anti-coagulant, the bottle was gently shaken clockwise and anti-clockwise to ensure adequate mixing of the blood with EDTA to prevent coagulation and the remaining 2.5 ml was poured into another sample bottle without EDTA. The following parameters such as packed cell volume, haemoglobin, red blood cell, white blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, monocytes, lymphocytes, neutrophils, total protein, albumin, globulin, albumin/globulin ratio, glucose, aspartate aminotransferase and alanine aminotransferase were assessed using standard methods as described by (Henry, 1964; Trinder, 1969; Tietz*et al.*, 1983)

Statistical Analysis

Data obtained from the experiment were subjected to one-way Analysis of Variance (ANOVA) using SPSS 20.0 version. Where the ANOVA revealed significant differences ($P<0.05$), Duncan's New Multiple Range Test was used to compare differences among individual treatment means.

Results

Proximate Composition of Experimental Diet

The proximate composition of the experimental diets used for the feeding trial is presented in Table1. Highest moisture content was recorded in SBL_3 (2%) and lowest in $(SBL+ERY)_5$, the highest value of crude protein was recorded in SBL_3 (2%) and lowest in the control diet. The ash value obtained revealed that $(SBL+ERY)_5$ recorded the highest value and least in control

Growth Performance and Nutrient Utilization of *C. gariepinus* fed Experimental Diet

The result shows that the experimental fish utilized the diets. The highest weight gain was recorded in SBL_2, followed by $SBL +ERY_5$ and the lowest in ERY_4. The highest specific growth rate was obtained in the treated group, SBL_2 and the lowest in the positive control. The value of protein productive value and protein efficiency ratio recorded were not significantly different ($P > 0.05$) among the dietary groups while the value of feed conversion ratio and feed efficiency ratio shows the significant difference ($P< 0.05$) among the dietary groups (Table 2).

Table 2: Growth performance and nutrient utilization of *C. gariepinus* fed the experimental diet for 4 weeks

	Control	SBL_2 (1%)	SBL_3 (2%)	ERY_4 (30mg/kg)	$(SBL + ERY)_5$ (2%)
IBW	6.32±0.01 ^{a}	6.32±0.02 ^{a}	6.32±0.01 ^{a}	6.32±0.00 ^{a}	6.32±0.02 ^{a}
FBW	8.67±0.00 ^{b}	8.90±0.03 ^{d}	7.97±0.02 ^{b}	7.56±0.01 ^{a}	8.69±0.00 ^{c}
WG	2.35±0.01 ^{c}	2.58±0.04 ^{d}	1.65±0.03 ^{b}	1.24±0.00 ^{a}	2.37±0.01 ^{c}
PWG	37.18±0.03 ^{c}	40.82±0.06 ^{e}	26.11±0. 05^{b}	19.62±0.02 ^{a}	37.50±0.02 ^{d}
SGR	0.49±0.02 ^{c}	0.53±0.01 ^{c}	0.36±0.00 ^{b}	0.28±0.01 ^{a}	0.49±0.01 ^{c}
PPV	0.44±0.03 ^{a}	0.51±0.06 ^{a}	0.46±0.01 ^{a}	0.46±0.02 ^{a}	0.49±0.01 ^{a}
PER	0.05±0.01 ^{a}	0.06±0.02 ^{a}	0.04±0.01 ^{a}	0.03±0.00 ^{a}	0.05±0.00 ^{a}
FCR	1.75±0.04 ^{d}	1.67±0.09 ^{c}	1.36±0.10 ^{b}	0.97±0.03 ^{a}	0.94±0.05 ^{a}
FER	0.57±0.01 ^{a}	0.60±0.03 ^{a}	0.73±0.08 ^{b}	1.03±0.10 ^{c}	1.06±0.09 ^{c}

IBW= Initial body weight, FBW= Final Body Weight, SGR= Specific Growth Rate, PWG= Percentage Weight Gain, WG= Weight Gain, PER= Protein Efficiency Ratio, PPV= Protein Productive Value, FCR= Feed Conversion Ratio, FER= Feed efficiency Ratio

Haematological parameters of experimental fish fed *P. niruri* leaf meal-based diets

There was an increase in the value of haematological parameters of the post-challenge test compared to the pre-challenge value and control. Fish fed (SBL+ ERY) $_5$ recorded the highest value in PCV, HB, RBC and WBC, there were significant difference (p>0.05) among the dietary groups and the lowest value was obtained in the pre-challenge and control. The values obtained for lymphocytes in treated groups were higher (P<0.05) when compared with pre-challenge and control. Among the groups, a combination of (SBL+ ERY) $_5$ shows a synergistic effect with a relatively higher value among the parameters assayed.

Table 3: Mean post Challenge test Haematological Parameter of African Catfish *C. gariepinus* fingerlings fed with treated Stone breaker leaves

Parameter	Before	Control	SBL_2 (1%)	SBL_3 (2%)	ERY_4	$(SBL+ERY)_5$
PCV (%)	42.00±0.00^{b}	41.00±0.50^{a}	43.00±0.50^{c}	45.00±0.50^{d}	42.00±0.00^{b}	46.00±0.00^{e}
HB (g/l)	14.00±0.00^{a}	14.70±0.00^{abc}	14.30±0.00^{ab}	15.00±0.50^{bc}	14.00±0.50^{a}	15.30±0.00^{c}
RBC (m/l)	4.80±0.50^{ab}	4.80±0.50^{ab}	4.85±0.00^{ab}	4.90±0.50^{ab}	4.50±0.00^{a}	5.30±0.00^{b}
WBC (m/l)	10.80±0.50^{a}	11.50±0.00^{b}	12.70±0.00^{c}	11.60±0.50^{b}	10.71±0.00^{a}	11.8±0.50^{b}
Platelet (mcl)	24.70±0.00^{d}	19.40±0.00^{a}	28.10±0.50^{f}	21.8±0.00^{b}	23.60±0.00^{c}	27.30±0.00^{e}
MCV(fl)	87.50±0.50^{b}	85.41±0.00^{c}	88.66±0.50^{b}	91.83±0.00^{c}	93.33±0.00^{d}	86.79±0.50^{a}
MCH (pg/cell)	29.16±0.00^{b}	30.62±0.00^{c}	29.48±0.50^{b}	30.61±0.00^{c}	31.11±0.00^{c}	8.87±0.50^{a}
MCHC (g/dl)	33.33±0.00^{a}	35.86±0.00^{a}	33.26±0.00^{a}	33.33±0.00^{a}	33.33±0.00^{a}	33.26±0.00^{a}
NEUT (%)	65.00±0.50^{d}	64.00±0.50^{c}	60.00±0.50^{a}	66.00±0.50^{e}	63.00±0.50^{b}	78.00±0.50^{f}
LYMP (%)	32.00±0.50^{a}	32.00±0.5^{a}	35.00±0.5^{bc}	36.00±0.05^{c}	34.00±0.50^{b}	36.00±0.50^{c}
MONO (%)	3.00±0.50^{c}	3.00±0.50^{c}	5.00±0.50^{d}	2.00±0.50^{b}	2.00±0.50^{b}	5.00±0.00^{a}
EOS (%)	0.00±0.00^{a}	1.00±0.05^{b}	0.00±0.00^{a}	1.00±0.05^{b}	1.00±0.05^{b}	0.00±0.00^{a}

Means in the same row with similar superscripts are not significantly different (p>0.05), SBL= Stone breaker leaves, ERY= Erythromycin, PCV=Packed cell volume, Hb= Haemoglobin, RBC= Red blood cell, WBC=White blood cell, MCV=Mean cell volume, MCH=Mean cell haemoglobin, MCHC=Mean cell haemoglobin concentration, Lym=Lymphocytes, Mono=Monocytes, Eos=Eosinophils.

Blood Biochemical

Result of blood biochemical obtained in this study is presented in table 4. SBL_3 recorded the highest value (78.00±0.50) in total protein and fish fed control recorded the lowest value (70.00±0.20). There was a significant difference (P>0.05) among the dietary groups. Fish fed with diet SBL_2 (1%) recorded the highest value of albumin (48.00±0.50) and the lowest value (39.00±0.30) was obtained in ERY_4. The value of globulin recorded in SBL_3 had a higher value among the dietary groups and lowest in SBL_2. There was no significant difference (P>0.05) in albumin-globulin among the dietary groups. The result of Amino Alanine Transferase (ALT) revealed a decrease in the value obtained among the dietary groups (P<0.05) and the values of Aspartate Amino Transferase (AST) increase among the dietary groups, there was significantly different (P <0.05) among the dietary groups.

Table 4: Blood biochemical Parameters of African Catfish *C. gariepinus* fingerlings fed Stone breaker leaves

Parameter	Before	Control	SBL_2 (1%)	SBL_3 (2%)	ERY_4	(SBL+ERY) $_5$
Total Protein (g/l)	73.00±0.50 ^{b}	70.00±0.20 ^{a}	73.00±0.40 ^{b}	78.00±0.50 ^{c}	73.00±0.00 ^{b}	72.00±0.30 ^{b}
Globulin (g/l)	34.00±0.40 ^{d}	30.00±0.50 ^{b}	25.00±0.00 ^{a}	38.00±0.50 ^{e}	34.00±0.10 ^{d}	32.00±0.00 ^{c}
Albumin (g/l)	39.00±0.50 ^{a}	40.00±0.10 ^{b}	48.00±0.50 ^{c}	40.00±0.40 ^{b}	39.00±0.30 ^{a}	40.00±0.50 ^{b}
A:G Ratio(g/l)	1.20±0.00 ^{a}	1.30±0.00 ^{a}	1.50±0.00 ^{a}	1.30±0.50 ^{a}	1.20±0.00 ^{a}	1.30±0.00 ^{a}
AST	12.0±0.30 ^{c}	10.00±0.50 ^{a}	11.00±0.20 ^{b}	12.00±0.10 ^{c}	12.00±0.50 ^{c}	10.00±0.20 ^{a}
ALT	14.00±0.20 ^{b}	13.00±0.50 ^{ab}	12.00± 0.10 ^{a}	11.95±0.50 ^{a}	13.00±0.30 ^{ab}	13.00±0.10 ^{ab}
Glucose (Mmol/l)	9.80±0.20 ^{c}	7.90±0.10 ^{ab}	9.80±0.40 ^{c}	8.40±0.00d ^{b}	8.20±0.00 ^{b}	7.40±0.00 ^{a}

Keys: A/G Ratio= Albumin/globulin ratio, ALT=Amino Alanine Transferase, AST=Aspertate Amino Transferase. Mean of triplicate data, the mean value in each row with similar superscripts are not significantly different (p>0.05).

Challenge Test

The result of the study shows that the treated groups had lower mortality percentage when compared to the control group. The relative percentage of survival was higher in the treated groups than the control groups and there was a significant difference (P< 0.05) among the dietary groups (Table 5).

Table 5: Challenge test of *P. aeruginosa* injected by the intraperitoneal route and relative percentage of survival among *C. gariepinus* fingerlings treated with Stone breaker

	Control	SBL_2 (1%)	SBL_3 (2%)	ERY_4 (30mg/kg)	SBL + ERY $_5$ (2%)
No of injected fish	15^{a}	15^{a}	15^{a}	15^{a}	15^{a}
Mortality (N)	11^{b}	1^{a}	0^{a}	3^{a}	0^{a}
Mortality (%)	73.33±0.06 ^{d}	6.67±0.02 ^{b}	0±0.00 ^{a}	20±0.02 ^{c}	0±0.00 ^{a}
RPS	0±0.00 ^{a}	91±0.01 ^{c}	100±0.00 ^{d}	73±0.02 ^{b}	100±0.00 ^{d}

Means in the same row with similar superscripts are not significantly different (p>0.05)

Discussion

The crude protein content of the experimental diet recorded in this study supports the growth of *C. gariepinus* fingerlings which ranged (28-50% CP) requirements for catfish as reported by Chukwukadibia, (2016). This study revealed that the treated groups recorded significantly higher value (P>0.05) of crude protein and ash content when compared to the control group. This could be due to the presence of plant additives such as *P. niruri* present in the diets.

The result of the study revealed that the diet was better utilized by the experimental fish for growth and development. The treated groups recorded better growth performance than the control group. The diet supplemented with 1% *P. niruri* performed better than other inclusion levels and the control. The higher value obtained in the treated groups could be due to the availability of growth enhancer in *P. niruri* (flavonoids, alkaloids and tannins). The result of this study was in accordance with the report of Olusola *et al.*, (2020) who reported better performance in term of weight gain, specific growth rate, feed conversion ratio, protein productive value, and nitrogen metabolism in the treated groups compared to the control group of *C. gariepinus* juveniles fed tamarind and mango leaves. Also, Abou-zeid, (2002) reported that inclusion of *Allium sativum* in the diet of *Oreochromis niloticus* increases the weight gain and specific growth rate which was similar to the findings of this study.

Haematological indices were important indicators of the physiological status of animal and changes in the values are used in assessing the response of the animal to various conditions (Esonu*et al.*, 2006). The result of the study (table 3) shows that the value obtained range (41.00±0.50 - 46.00±0.00) for Packed Cell Volume (PCV) and was within the normal range of 40 – 54 as reported by Jordan, (1992). Packed cell volume and haemoglobin is used as an indicator of the nutritional status of the animals (Adejumo, 2004). Increase in PCV of this study shows better transportation of oxygen and absorbed nutrients, this result in increased primary and secondary polycythemia. This result showed that there was a concordance in the study report of Olusola *et al.*, (2020) who reported that PCV had higher numerical value in the treated groups of post-challenge than the control group and pre-challenge of *C. gariepinus* juveniles fed tamarind (*Tamarindus indica)* and mango (*Mangifera indica)* leaves. The red blood cell values (4.50±0.00 -5.30±0.00) in the present study were within (4.7 – 6.1) recommended by Jordan, (1992) for fish. The white blood cell recorded in the treated groups was higher than the control.

The study revealed higher values of haemoglobin in the treated groups expect SBL 1% compared to the control. An increase in haemoglobin is an important determinant of possibilities of no anaemia, this resulted in an increase in oxygen carry capacity of blood in *C. gariepinus* fingerlings with performance improvement. This present study does not agree with Das *et al.,* (2009) who reported a decrease in Hb after 10 days of the challenge with *Aeromonas hydrophila* in *Labeo rohita*. Although the values reported in this present study showed no significant difference (P>0.05) in Hb but recorded value higher than the pre-challenge and the control. The higher WBC count of *C. gariepinus* fingerlings on treated diets compared to the control could be due to the presence of phytochemical constituents (alkaloids, saponins and flavonoids) present in *P. niruri*. This further supported by the high lymphocytes count in the treated groups compared to the control. This observation revealed that the treated

diets enhance haemopoietic tissues with the production of adequate WBC. White blood cell functions as a defence system as it contains lymphocyte that has a central role in the immunological defence mechanism of the body.

Generally, there was an increased value of lymphocytes obtained among the treated groups which recorded higher value than the pre-challenge and control and the value was significantly different (P<0.05) among the dietary groups. The values recorded in the control and pre-challenge test were lower than those of the treated groups, the reason for this might be due to lower immune functions against the fish pathogen, *P. aeruginosa*. The present study corroborates the report of Bello *et al.,* (2012) who recorded higher value of lymphocyte and white blood cell in treated groups when compared with the control and pre-challenge test of *C. gariepinus* fed *Tetracarpidium conophorum* leaf and *Allium cepa* bulb against *P. aeruginosa*. The low values of eosinophils and monocytes recorded in this study agree with the report of Odeys, (1996) who reported that eosinophils and monocytes normally present in small to moderate numbers in the peripheral blood system of an animal.

The value of total protein obtained in the study was higher in the treated groups compared to the control and these values were within the normal values (60 – 80) reported by Jordan, (1992) for fish. An increase in the total protein is an indication of the presence of good quality protein in the diets and good quality protein utilization. The values obtained in control and treated groups for albumin were higher than the pre-challenge. There was a significant difference (P>0.05) among treatments. This present study was similar to the report of Olusola *et al.*, (2020) who reported higher value of albumin in control and treated groups compared to the pre-challenge of *C. gariepinus* juvenile fed *Tamarindus indica* and *Mangifera indica* leaves. The values of albumin and globulin ratio recorded in the study show a similar trend but the highest numerical value was obtained in SBL 1% inclusion. Bello *et al.,* (2012) recorded an increase in values of albumin and globulin ratio of treated groups as compared to the control and pre-challenge on *C. gariepinus* juveniles fed walnut leaves and onion bulb which also shows similarity with the present study.

The reduction in alanine aminotransferase (ALT) of the treated groups can be an indication of better liver functions and possibilities of ruling out anorexia with better bone formation. The values of aspartate aminotransferase (AST) content of the treated groups were significantly increased compared to the control. The treated groups recorded lower values of ALT. This study was in accordance with the report of Imaseun and Ijeh, (2018) in which they reported an increase in the value of AST and reduction in the value of ALT in birds fed medicinal plants (ginger and black pepper diet). Bello *et al.*, (2012) also reported a decrease in ALT value *C. gariepinus* fingerling fed walnut leaves and onion bulb at different inclusion levels which agree with the present study.

Relative percentage of survival (RPS) was 100% in SBL_3 (2%), $(SBL+ERY)_5$ and 0% in control. There was a significant difference (P < 0.05) in RPS among the dietary groups. This is an indicator that these plants have non-specific immune-stimulants that enhance the immunity of *C. gariepinus* fingerlings against pathogen *P. aeruginosa* and It can be inferred from the challenge test study that the increase RPS of the injected fish could be due to the enhancement in the defence system emanating from the increase in the value of immunes such as lymphocytes and white blood cell recorded in table 3.

Thirty minutes after injection, a clinical sign

such as abnormality in swimming was observed and ulcerative lesion on the skin, oedema (swollen belly near the heart) and mortality after some days were also observed. This present study agrees with some of the identified signs reported by Olusola and Nwokike, (2018). The mortalities percentage was lower in the treated groups compared to the control. This study corroborates the report of Shalaby *et al.*, (2006) who reported that diet with *Allium sativum* and chloramphenicol showed a decrease in the mortality rate of *O. niloticus* challenged with *Aeromona hydrophila.* Also, Sharma *et al.*, (2010) reported a decrease in mortalities percentage of a fish fed medicinal plant when compared with the control.

Conclusion

Results of this study showed that *P. niruri* leaves meal-based diets is a good potential unconventional feed ingredient that could be used in aquaculture to stimulate immune response as well as prevent the infection of *P. aeruginosa* in *C. gariepinus* fingerlings. It is therefore recommended that inclusion of *P. niruri* in the diets of *C. gariepinus* would improve productivity in aquaculture. Further research should be carried out to explore the potential of *P. niruri* on other species of fish.

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