Phyto-Prophylactic Potentials of Dietary *Ipomoea-batatas* Leaves on *Clarias gariepinus* Exposed to *Psedomonas aeruginosa:* Biochemical Analysis and Liver Histopathology

ABSTRACT

Ipomoea batatas aqueous extracts was examined for it's phyto-prohylatic effects on the enzymes, bilirubin, metabolites, electrolytes and liver histopathology of *Clarias gariepinus* challenge with *Pseudomonas aeruginosa*. One hundred and fifty (150) *C. gariepinus* were distributed into five groups in triplicates and fed accordingly with 0ml/kg (DO), 50ml/kg (D1), 100ml/kg (D2) and 150ml/kg (D3*) I. batatas* supplemented diets. Two groups were fed with 0ml/kg ($D0^{+ve}$ and $D0^{-ve}$). At the end of the feeding period, the fish fed $D0^{-ve}$ (negative control) and D1-D3 were infected with 1.5ml if 1.5×10^{10} cfu/ml overnight grown *Pseudomonas aeruginosa* while the fish fed $D0^{+ve}$ was unfected. After seven (7) days post infection period, blood samples were collected from all groups to determine enzyme activities [Aspartate aminotransferase (AST), Alanine transaminase (ALT) and (ALP),] Bilirubin activities [Total Bilirubin (TB) and Conjugated Bilirubin (CB)], Metabolites activities [Total Protein (TP), Albumine (ALB), Globulin (GLB), Urea (UR) and creatinine (CR)] and Electrolytes activities [Sodium (Na^+), Potassium (K^+), calcium (Ca^{2+}), chloride (CL^-) and bicarbonate (HCO^-_3)]. The fish livers were also harvested for histopathological analysis. The results revealed that the highest values of all enzymes and bilirubin activities were recorded in the fish fed D^{-ve} (negative control) when compared to the fish fed D1 to D3 (treated groups) and D^{+ve} (positive control). TB and GLB were lower in the fish fed D^{-ve}, UR and CR were higher in the fish fed D^{-ve} when compared to the fish fed D1-D3 and D^{+ve}. ALB values were similar in the fish fed D^{+ve} and D3 ($P<0.05$). Na^+ and CL^- were significantly lower in the fish fed D^{-ve} and the highest values of Ca^{2+} and K^+ were also recorded in the fish fed D^{-ve}. However, the values of HCO^-_3 had no significant difference. The histopathological analysis shows that the liver of the fish fed D^{+ve}, D2 and D3 had exact same features which are suggestive of a cultured fish. However, the liver of the fish fed D-ve and D1 had no vacuoles and there was a mild infiltration by lymphocytes in the liver of the fish fed D1. Sequel to these results, it is evidential that *I. batatas* possess the potential to maintain normal biochemical activities, enhance survival rate, resist disease and safeguard fish liver against *P. aeruginosa*. It is recommended that aquaculturist should embrace and apply this great discovery to maintain sustainability.

Keywords: Pseudomonas aeruginosa, histopathological analysis, fish fed, animal protein

2. INTRODUCTION

Fish and fish products are relatively cheaper compared to other sources of animal protein like beef and pork (1). Fish is a less expansive and very significant source of protein and it supplies the body with other essential nutrients. There is a serious decline in fish supply from the wild as a result of pollution arising from industrialization and other forms of development (2), and there is the need to culture fish outside its natural environment. *Clarias gariepinus* is the most cultured fish species in Africa, and it is considered most suitable to culture outside its natural environment (3).

However, one of the major challenges facing fish farmers is the occurrence of diseases (4). The progression of aquaculture is hindered most times by virus, bacteria, parasites, fungi and some environmental interactions, leading to an unhealthy and stressful environment that causes the suppression of the immune system and increasing the susceptibility of fish to infectious diseases (5,6). Fish diseases tend to spread quickly through the water and this is a source of great concern to those in the practice of aquaculture, (7). One of the infectious bacteria that causes diseases in African catfish is *Pseudomonas aeruginosa* (8).

Fish disease can easily lead to economic losses and the spread of diseases to consumers. The adequate management of diseases is crucial for the continuous expansion of the aquaculture industry since disease presence can cause severe morbidity and mortality of fish stocks (9). Over the years, antibiotics and other veterinary synthetic drugs are administered regularly as additives in fish feed as prophylactics (preventing diseases before they occur), therapeutics (treating sick animals) or growth promoters (10) but many nations have restricted the use of these synthetic drugs because they possess serious risks on the environment and humans (11). The remains of antibiotics accumulate in the tissue of fish and significantly increases pathogen resistance andpollute the environment. (17, 13). Herbs and herbal products have proven to be a good solution and a replacement for synthetic drugs in the practice of aquaculture as they are eco-friendly, not immune-specific and does not deposit on the fish flesh. (4).

The presence of diseases and their causative pathogens can be detected in fish in various ways including analysis of biochemical parameters (14, 15), histopathological and hematologicalassessment (16, 6).

Ipomoea batatas (sweet potato) leaf possess significant nutrients and phytochemicals that can enhance productivity in aquaculture (17). The purpose of this research is to access the prophylactic properties of *I. batatas* in the development of aquaculture.

3. MATERIALS AND METHODS

3.1 Study Location

The research was carried out in the laboratory of the Fisheries and Aquatic Environment Department, Faculty of Agriculture, Rivers State University, Nkpolu – Oroworukwo, Port Harcourt.

3.2 Experimental Fish and Acclimation

One hundred and fifty (150) healthy *C. gariepinus* of mean weight 130-150kg was purchased from Idi-onyana Farms along Abua-Ahoada Road in Abua/Odual Local Government Area, Rivers State. The fish was taken to the study location, acclimatization and observation was carried out on the fish for a period of fourteen (14) days to evaluate disease presence or bruises. During this period the fish was fed to satiation with blue crown commercial feed twice daily.

3.3 Determination of Water Quality Parameters

Temperature, dissolved oxygen (DO) and pH were determined using thermometer, DO meter and pH meter respectively.

3.4 Source of Pathogen

The pathogen *Pseudomonas aeruginosa* was ordered from the National Veterinary Institute, Vom in Jos, Plateau State, Nigeria and was transferred to the Department of Microbiology of the Rivers State University, Nkpolu Oroworukwo, Port Harcourt for preservation.

3.5 Preparation of *Ipomoea batatas* Leaf extracts

The Sweet potato (*Ipomoea batatas*) leaves were harvested within Port Harcourt, Rivers State, Nigeria. The leaves were prepared using the method of (8). *I. batatas* leaves were washed clean, pound to paste, soak in tap water (50°C) at the concentration of five hundred grams/litre (500g/L). It was filtered and the filtrate was used immediately.

3.6 Experimental Diet

35% CP (crude protein) feed was formulated using the following ingredient: Wheat-bran, corn, soyabeans, fish meal, lysine, methionine, oil, starch and vitamins C. Four (4) different diets were produced from the formulated feed using varying quantities of *I. batatas* leaves extract as follows: 0ml/g, 50ml/kg, 100m/kg and 150ml/kg and labelled D0, D1, D2 and D3 respectively.

3.7 Experimental Design

The research design adopted for this research was a complete randomized method (CRD). A total of fifteen (15) experimental tanks were used in the experiments. There were five treatments in triplicates.

3.8 Experimental Procedure

One hundred and fifty (150) *C. gariepinus* were distributed into five groups in triplicates, and acclimatized for 2 weeks. They were distributed into fifteen (15) 50 - litres tanks at 10 fish per tank. Feeding commenced 24 hours after stocking. Two groups were fed D_0, and labeled D_0^{-ve} (negative control)and D^{+ve} (positive control), while the three other groups were fed D1 D3 and labeled accordingly as D1 – D3. At the end of the feeding period, the fish fed (Do)

and D1 – D3 were infected intraperitoneally with 1.5ml of 1.5 x 10^{10} CFU/mL overnight grown *P. aeruginosa*, while the fish fed Do^{+} were uninfected,andwere all observed for 7 days. After seven (7) days post infection period, blood samples were extracted from the experimental fish across the groups (in the replicates) and taken to the laboratory for biochemical analysis, and the liver were harvested and taken to the laboratory for the determination of liver histopathological analysis, while water quality was assessed before and after water exchange daily.

3.9 Blood Extraction

The fish was blind folded by covering the head with a thick cloth to attain calmness (18) and blood was extracted via kidney puncture through the genital opening using 5ml injection syringe.

3.10 Enzymes Analysis

After seven (7) days post-infection period, blood samples were randomly collected from three fish in each group via kidney puncture, using 5 ml injection syringe and niddle. The collected blood samples were transferred into lithum heparin tube and sent to the laboratory for biochemical analysis within twelve (12) hours. They were assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB). This was done by the use of "Evolution 3000 machine" an auto-analyzer, the screen master model, manufactured by Biochemical system, China. It was used according to manufacturers instructions.

3.10 Electrolytes Analysis

The electrolytes, sodium (Na^{+}), potassium (K^{+}), Calcium (Ca^{+2}), and chloride (Cl^{-}) levels were determined using the automatic analyzer and optimal test by means of flame photometry as described by Schales and Schales (1941)

3.12 Metabolite Analysis

The blood samples were centrifuged using a centrifuge 80-2 machine, manufactured by Technical and Technical in United States of America. The sample were centrifuged at 4000rpm for fifteen (15) minutes to separate the plasma and put in a curvettte. Spectrophotometric analysis was performed on the blood plasma using a spectrephotometer, model "SURGISPEC SM – 230" manufactured by surgifield medical in England and was used according to manufacturers instruction, as stated in (19).

3.13 Histopathological Analysis

The fish liver was taken to the laboratory in sample bottles containing 10% formalin solution. The samples (liver) were manually processed and trimmed using a rotary microtone (LEICA RM 2125 RTS), manufactured by LEKA Brosysteo, Buffalo Grove, U.S.A. Tissues were dewaxed, stained in hematoxylin and eosin for a display of tissue architecture. Stained slides were examined under light microscope at x 10 magnification.

3.14 Data Analysis

The collected data were analyzed using SPSS statistics software 17.0 windows. A one-way analysis of variance (ANOVA) was employed to reveal a significant difference between control and treated groups. Tukey's multiple comparison test was applied to separate treatments with significant differences (20)

RESULTS

4.1 Physicochemical Parameters of the Experimental Waters

The result of the water quality parameters wereshown in Table .1. There were no significant difference in all the determined water quality parameters.

4.2 Enzymes and Bilirubin Activities in the Plasma Biochemistry of the Experimental Fish after Seven (7) days of Exposure to *Pseudomonas aeruginosa*.

The values of enzymes and Bilirubin activities in the plasma biochemistry of the experimental *C. gariapinus* after 7 days of exposure to *_P. aeruginosa*are presented in Table 2. The results indicated that there were significant differences in the values of aspartate aminotransferase (AST). The values for the fish fed experimental diets D^{+ve} (36.00±4.58) D2 (38.33±7.63) and D3 (39.00±8.88) were significantly lower while value for the fish fed D^{-ve} was higher (47.33± 2.08) and the experimental fish fed D1 had the highest value of AST. (40.00±7.21). There were no significant differences in the values of Alanine transaminase (ALT) for the fish fed the various experimental diets. There were significant differences in the values of Alanine Phosphate (ALP). The experimental fish fed D^{+ve} (59.00±13.85) was significantly different from all other experimental fish. However, the highest ALP values were recorded in the experimental fish fed diets D^{-ve} (66.33±15.30) and D3 (63.66±29.14), while the lowest values were recorded in experimental fish fed diets D1 (44.66±16.28) and D2 (44.66±26.38). For Total bilirubin, the experimental fish fed diets D^{+ve}, D1 and D3 had similar values (7.26±0.56 - 7.43+ 1.20). However, lower value of TB was recorded in experimental fish fed D2 (6.93± 0.37) while the experimental fish fed diet D^{-ve} had the highest TB value (8.46±0.55). The Conjugated Bilirubin (CB) values were within the same range in the experimental fish fed diets D^{-ve} (5.73±0.45) D2 (5.23±2.72) and D3 (5.13±2.65), but significantly lower in the experimental fish fed diet D^{+ve} (4.66 ±0.56) and D1 (4.63±0.98). `

Table 1: Water Quality Parameters of the Experimental tanks (Means ±)

	Parameters		
Experimental Diets	**Dissolve oxygen**	**Temperature (^{0}C)**	**pH**
D_{+v}	5.03±0.13[b]	28.13±0.15 [a]	7.40±0.93 [a]
D_{-ve}	3.09±0.35 [a]	28.05±1.15 [a]	66.3±0.15 [a]
D_1	4.90±0.56 [b]	28.25±0.91 [a]	7.13±0.78 [a]
D_2	4.13±0.38 [b]	28.10±0.36 [a]	7.34±1.41 [a]
D_3	4.17±1.31 [b]	27.91±0.31[a]	6.98±0.17 [a]

Means within the same column with different superscripts are significantly different (P<0.05)

Table .2: Enzymes and Bilirubin Activities in the Plasma Biochemistry Experimental Fish after Seven days of Exposure to *P. aeruginosa*(Mean ±SD)

Experimental Diets	Parameters				
	AST(IU/L)	**ALT(IU/L)**	**ALP(IU/L)**	**TB(Umol/L)**	**CB(Umol/L)**
D_{+VE}	36.00±4.58[a]	24.00±1.00 [a]	59.00±13.85 [b]	7.43±0.50 [b]	4.66±0.56 [a]
D_{-VE}	47.33±2.08 [c]	27.66±4.72 [a]	66.33±15.30 [c]	8.46±0.55 [c]	5.73±0.45 [b]
D_1	40.00±7.21 [b]	24.66±3.51 [a]	44.66±16.28 [a]	7.43±1.20 [b]	4.63±0.98 [a]
D_2	38.33±7.63 [a]	25.33±3.21 [a]	44.66±26.38 [a]	6.93±0.37 [a]	5.23±2.72 [b]
D_3	39.00±8.88 [a]	24.66±4.72 [a]	63.66±29.14 [c]	7.26±0.56 [b]	5.13±2.65 [b]

Means within the same column with different superscripts are significantly different (P<0.05) Key: AST: Aspartate aminotransferase; **ALT:** Alanine transaminase; **ALP:** Alkaline Phosphate; **TB:** Total Bilirubin; **CB:** Conjugated Bilirubin

4.3 Metabolite Activities in the Plasma Biochemistry of the Experimental Fish after Seven (7) days of Exposure to *P. aeruginosa.*

The values for metabolite activities in the plasma biochemistry of the experimental *C. gariapinus* after 7 days of exposure to *P. aeruginosa*are presented in Table .3. The results indicated that the Total Protein (TP)value for the experimental fish fed D^{+ve} (51.66±24.98) D2 (54.00±8.18) and D3 (53.00±7.93) were within the same range but significantly lower in experimental fish fed D^{-ve} (44.00 ±8.54) and higher in experimental fish fed D1 (59.33±18.00). Albumin values were similar in the experimental fish fed diets D^{-ve} (25.00±2.64), D1 (25.33±8.38) and D2 (23.33±6.11) but significantly higher in experimental fish fed D3 (26.00±1.73) and D^{+ve} (27.66±10.26). Globulin values fluctuated across the experimental fish fed various diets but was significantly lower in fish fed D^{+ve} (19.00±8.71), followed by D^{-ve} (24.00±14.73), fish fed diets D2 and D3 were within same range (27.00+8.66 - 30.66±19.15) while the value for the fish fed diet D1 was significantly higher (34.00±19.15). Urea (UR) values were significantly the same for the fish fed diets D^{+ve}, D1 and D3 but higher in fish fed D^{-ve} (3.43+0.40) and D2 (3.10±0.78). Creatinine (CR) values were similar for the experimental fish fed diets D^{+ve} (55.33±23.02) and D1 (56.33±13.61), followed by the experimental fish fed diets D2 (62.00±27.78) and D3 (61.66±16.19) but significantly higher in experimental fish fed diet D^{-ve} (74.33±2.08).

Table .3: Metabolites Activities in the Plasma Biochemistry Experimental Fish after Seven days of Exposure to *P. aeruginosa*

Experimental Diets	PARAMETERS				
	TP (g/l)	**ALB(g/l)**	**GLB(g/l)**	**UR (Muol/L)**	**CR(Muol/L)**
D_{+VE}	51.66±24.98[b]	27.66±10.26[b]	24.00±14.73[b]	2.93±0.55[a]	55.33±23.02[a]
D_{-VE}	44.00±8.54[a]	25.00±2.64[a]	19.00±8.71[a]	3.43±0.40[b]	74.33±2.08[c]
D_1	59.33±18.00[c]	25.33±8.38[a]	34.00±19.15[d]	2.70±0.55[a]	56.33±13.61[a]
D_2	54.00±8.18[b]	23.33±6.11[a]	30.66±4.93[c]	3.10±0.78[b]	62.00±27.78[b]
D_3	53.00±7.93[b]	26.00±1.73[b]	27.00±8.66[c]	2.86±0.83[a]	61.66±16.19[b]

Means within the same column with different superscripts are significantly different (P<0.05)

Key: **TP:** Total Protein; **ALB:** Albumin; **GLB:** Globulin**; UR:** Urea; **CR:** Creatinine; **A /G:** Albumin-to-globulin;

4.4 Electrolytes Activities in the Plasma Biochemistry of the experimental fish after Seven (7) days of exposure to *P. aeruginosa*.

The values of Electrolyte activities in the plasma biochemistry of the experimental *C. gariapinus* after 7 days of exposure to *P. aeruginosa* are presented in Table .4. The results indicated that there were significant differences in the values of Na^+, the experimental fish fed diet D^{-ve} and D1 were within same range (120.33±15.69 and 127.00±29.13 respectively), followed by the experimental fish fed diet D2 and D3 with values within same range (136.33±23.15 and 132.33±10.78 respectively). However the Na^+ value in the experimental fish fed D^{+ve} was significantly higher (153.33±4.72) than other experimental fish. The values for K^+ were significantly the same for the experimental fish fed diets D^{+ve} (4.13±1.76), D2 (4.16±1.15) and D3 (4.23±0.96) but higher and lower in experimental fish fed diets D^{-ve} (5.63±1.50) and D1 (3.76±1.36) respectively. The Ca^{2+} values were within same range for fish fed diets D^{-ve} (2.93±0.07), D3 (2.29±0.39) but significantly lower in fish fed D^{+ve} (1.93±0.14). The results for CL^- fluctuated across all experimental fish but it was significantly the same for fish fed diets D^{-ve} (61.66±30.13), D1 (68.66±2.08) and D2 (65.00±5.19). It was significantly higher in fish fed diets D3 (74.00±9.00) and D^{+ve} (82.00±14.00). There was no significant difference in the values of HCO_3^- in all experimental fish fed the various experimental diets.

Table .4: **Electrolytes activities in the plasma biochemistry experimental fish after Seven days of exposure to *P. aeruginosa*(Mean ±SD)**

Experimental Diets	Parameters				
	Na^+ (Mmol/L)	**K^+(Mmol/L)**	**Ca^{2+}(Mmol/L)**	**CL^-(Mmol/L)**	**HCO_3^-(Mmol/L)**
D_{+VE}	153.33±4.72^{c}	4.13±1.76^{b}	1.93±0.14^{a}	82.00±14.00^{c}	27.33±3.05^{a}
D_{-VE}	120.33±15.69^{a}	5.63±1.50^{c}	2.93±0.07^{b}	61.66±30.13^{a}	27.00±3.60^{a}
D_1	127.00±29.13^{b}	3.76±1.36^{a}	2.51±0.58^{b}	68.66±2.08^{a}	27.00±2.64^{a}
D_2	136.33±23.15^{b}	4.16±1.15^{b}	2.19±0.38^{b}	65.00±5.19^{a}	25.00±5.29^{a}
D_3	132.33±10.78^{b}	4.23±0.96^{b}	2.29±0.39^{b}	74.00±9.00^{b}	26.33±3.51^{a}

Means within the same column with different superscripts are significantly different (P<0.05)

Key: Na^+: Sodium Ion; **K^+:** Potassium Ion; **Ca2$^+$:** Calcium Ion; **CL^-:** Chloride Ion: $HC0_3^{-8}$

4.5 Liver Histopathology of the Experimental Fish after Seven (7) days of Exposure to *P. aeruginosa*.

The liver histopathology of the experimental fish after Seven (7) days of exposure to *P. aeruginosa* are shown in plates 1 A - 1E

Plate 1A.is the liver of the fish from group D^{-ve}, it has normal hepatocytes (blue) and normal central vein (black) × 200. Plate 1**B** is the liver of the fish in group D^{+ve} without infection, it has normal appearing glycogen vacuoles (blue) and normal central vein (blue) ×200. Plate 1**C** is the liver of the fish in group D1 (50ml/kg), it has normal appearing hepatocytes (blue) and normal central vein (black), and there is a mild infiltration by lymphocytes (green). Plate1**D** is the liver of the fish in group D2 (100ml/kg), it has normal appearing glycogen vacuoles (blue) and normal central vein (blue) ×200. Plate 1**E**, is the liver of the fish in group D3 (150ml/kg), it has normal appearing glycogen vacuoles (blue) and normal central vein (blue) ×200.

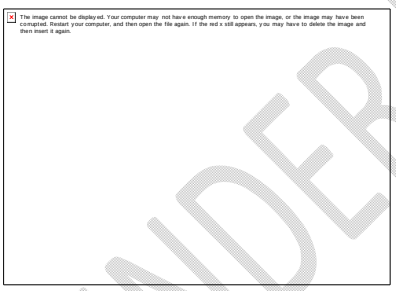

Plate 1A. Section shows normal appearing hepatocytes (blue) and normal central vein (black) X200

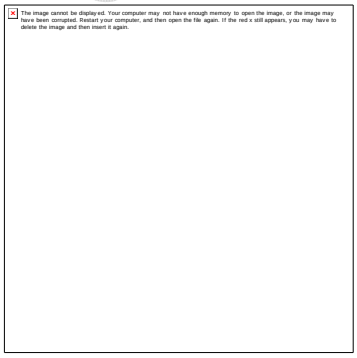

Plate 1B. Section shows normal appearing liver containing glycogen vacuoles (blue) which is suggestive of a cultured fish and normal central vein (black) X200

Plate 1C. Section shows normal appearing hepatocytes (blue) and normal central vein (black). There is mild infiltration by lymphocytes (green) X200

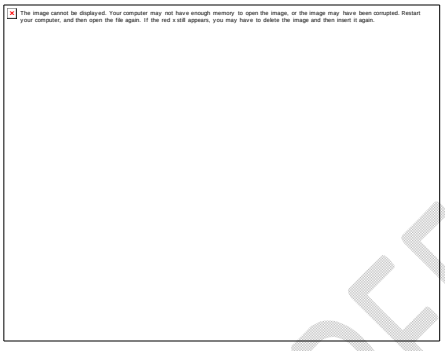

Plate 1D. Section shows normal appearing liver containing glycogen vacuoles (blue) which is suggestive of a cultured fish and normal central vein (black) X200

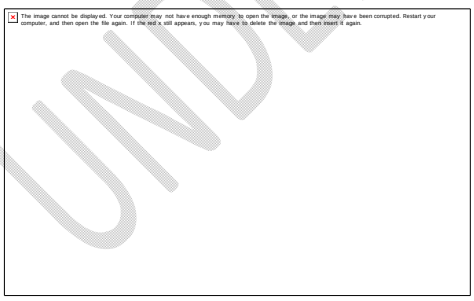

Plate1 E. Section shows normal appearing liver containing glycogen vacuoles (blue) which is suggestive of a cultured fish and normal central vein (black) X200

5. DISCUSSION

5.1 Effects of the *I. batatas* on the Enzyme and Bilirubin activities of *C. gariepinus infected* with *P. aeruginosa*

The result of the water quality parameters (temperature, dissolve oxygen and pH) reveals that the water quality in all the tanks (Table 1) supports the ultimate performance of fish (17,21). The reduction in dissolve oxygen in D^{-ve} could be as a result of lost of appetite in the fish fed the 0ml/kg diet and infected with the pathogen. The lost of appetite left uneaten feed, and decomposition of the uneaten feed led to the reduction in dissolve oxygen.

The use of medicinal herbs as immunostimulants in fish farming can boost the fish's natural defenses against pathogens (22). Enzymes such as aspartate aminotransferase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP), are biomarkers used to assess the health status of animals such as fish to ascertain the level on damage to the liver, heart, and other haematopoetic organs (18, 23, 24), and increase in these enzymes suggests the presence of disease (25). In this research work, the values of AST, ALT and ALP were lower in the fish from group D^{+ve}, D1, D2, and D3 compared to the group fed D^{-ve} after Seven days of exposure to *P. aeruginosa*. This result is in agreement with the findings of (19) who reported an increase in the activities of AST, ALT and ALP when *C. gariepinus* was infected with *P. aeruginosa* and exposed to *Zea Mays* (corn) husk extracts. (26), also reported increase in the activities of these enzymes when *C. gariepinus* was infected with *Staphylococcus aureus* and exposed to *Chromolaena odorata*. The significant increase in these enzymes (AST, ALT and ALP) in the Plasma of the fish in groups D^{-ve} (negative control) suggests that the experimental pathogen (*P. aeruginosa*) negatively affected the fish organs such as liver and kidneys (19, 26). However, the reduced activities of these enzymes (AST, ALT and ALP) in the Plasma of fish in group D1-D3 is a testament to the fact that the medicinal phytochemicals in *Ipomoea batatas* leaf extracts such as flavonoids, alkaloids, tennis,

surponin etc reduced the potency of *P. aeruginosa* from negatively affecting the liver and kidneys of the experimental fish (17). (6), also reported that these phytochemicals have the capacity to boost the immune system of *C. gariepinus* against *Klebsiella pneumonia*, and (17) also reported the prophylactic effect of *I. batatas* against*P. aeruginosae.*

Bilirubin is the primary by-product of the breakdown of red blood cells, and the liver is responsible for it's filtration and excretion from the body system making it a worthy biomarker of the liver's health (27). According to (28), high presence of bilirubin in the blood indicates abnormal break down of red blood cells or reduced absorption of bilirubin by the liver, and it is indicated by the presence of yellow pigmentation (jaundice). The result of the present study shows that the values of Total Bilirubin (TB) and Conjugated Bilirubin (CB) were significantly higher in the group D^{-ve} (negative control) compared to the other groups. This result is in consonant with the result of (19) who recorded increase in TB and CB when *P. aeruginosa* infected *C. gariepinus* was exposed to varying concentration of *Zea Mays* (corn) husk extracts. (29) also reported a similar observation when total conjugated bilirubin was studied in healthy of jaundice catfish. The increased presence of TB in the fish from D^{-ve} (negative control) could be as a result of inability of the liver to filter dead red blood cells from the fish body system (19), and increase in the CB level can be attributed to an obstruction in the bile duct (30). The reduced activities of TB and CB in the plsama of the fish in groups D1 to D3 can be attributed to the antibacterial properties of *I.batatas* leaf extracts against *P. aeruginosa*(31, 17).

5.2 Effects of the *I. batatas* on the Metabolite activities of *C. gariepinus infected* with *P. aeruginosa*

Plasma proteins (TP, ALB and GLB) are also biomarkers for disease presence in fish (32), and alterations in these metabolites in fish blood can suggest innate immune response or disease presence (32, 33). The result of this research reveals that total protein (TP) and

globulin (GLB) activities were lower in the fish from group D^{-ve} when compared to the fish in groups D1 - D3 after seven days of exposure to *P. aeruginosa*. This result supports the result obtained by (32) when *C. gariepinus* was fed dietary *Syzgium malaccence* aqueous leaves extract and exposed to *Staphylococcus aureus* for seven (7) days. ALB values for fish in group D^{+ve} and D3 were significantly the same but lower in fish in group D^{-ve}, D1 and D2. This result supports the findings of (34) who recorded lower ALB values after *Labeo fimbriatus* fingerlings were challenged with *Aeromonas hydrophila*. The significant decrease of these meatbolites (TP, ALB and GLB) in the fish from group D^{-ve} elucidates that the presence of *P. aeruginosa* may have caused tissue damage that stimulates protein synthesis as proteins aids tissue repair (35). However, their increase in the fish from group D1-D3 could be as a result of the medicinal phytochemicals present in *I. batata* leaf extracts that enhanced the fish immune system against the experimental pathogen (17). (36) also report antimicrobial activities of *I. batatas* leaves extract against *P. aerguniosa*.

Urea is a major product of the breakdown of proteins in the body (37) while creatinine is a major pointer to kidney functionality (38). UR and CR values were significantly higher in the fish from group D^{-ve} compared to the fish in other groups. This result agrees with the findings of (32) when the prophylactic potential of *Syzgium malaccence* aqueous leaves extracts against *Pseudomonas aeruginosa* was evaluated in catfish. The elevation of Urea and Creatinine in this study reveals the disfunctioning of the kidneys of the experimental fish (39). The decrease in UR and CR in the fish in groups D1-D3 could be as a result of the presence of antibacterial components in *I. batatas* leaf extracts that have reduced the potency of *P. aeruginosa* by boosting the immune system of the fish (17, 40), and it reveals the fact that dietary *I. batatas* leaves protected the kidney against *P. aeruginosa*.

5.3 Effects of the *I. batatas* on the Electrolytes Activities of *C. gariepinus infected* with *P. aeruginosa*

Alterations in Electrolytes are used as important biomarkers in the studies of aquatic animals (41) because they have osmoregulatory functions (42). The lowest values of Na^+ and CL^- were observed in the plasma of the fish in the group D^{-ve}. This result is in agreement with the result of (43) who recorded a decrease in Na+ and CL^- when the impacts of artificial *Aeromonas hydrophila* on hybrid catfish (*C. gariepinus* × *C. macrocephalus*) was studied. This significant decrease in Na^+ and Cl^- suggests that the experimental pathogen *P. aeruginosa* posed a negative effect on the physiology of the fish, resulting in the loss of these ions (44, 45).

The highest activities of Ca^{2+} and K^+ were recorded in the fish fed D^{-ve}. This result is in consonant with the result of (46) who reported increase in the activities of Ca^{2+} and K^+ when *Heterobranchus longigillis* was exposed to sub lethal levels of different chemicals in the laboratory. (47) also reported a similar result when the effects of water-soluble fraction of burnt tire-ash on *C. gariepinus* was investigated. These elevations suggests that the experimental pathogen (*P. aeruginosa*) damaged the permeability of the blood cell membrane (48). The reduced Ca^+ and K^+ in the fish in groups D1-D3 depicts that the medicinal phytochemicals in *Ipomoea batatas* leaf extracts were bacteriostatic or bactericidal to *P. aeruginosa*. The report of (49) supports this assertion.

There was no significant difference in the activities of HCO_3^- in all the experimental fish in the various group. This result supports the findings of (50) who reported the alterations of selected electrolytes in organs of *C. gariepinus.* The similarities of HC0 $HC0_3^-$ in all groups suggests that the experimental pathogen *P. aeruginosa* did not alter the respiratory system of the fish.

5.4 Effects of the Liver Histopathology of the Experimental *C. gariepinus after seven days exposure to P. aeruginosa*

Histopathological analysis determinings cellular changes that may occur in target organs such as gills, liver, spleen etc (52). After seven (7) days exposure to *P. aeruginosa*, the fish in groups D2 (Plate 1D) and D3 (Plate 1E) had normal appearing glycogen vacuoles (blue) and normal central vein (black) in their liver, similar observation was seen in this group D^{+ve} (Plate 1B).. These features are suggestive of a cultured fish. whereas the liver of the fish in groups D^{-ve} (Plate 1A) and D1 (Plate 1C) had appearing hepatocytes and normal central vein (black) and no glycogen vacuoles. This results is in consonant with the report of (16) when histological examination was carried out on the liver of *C. gariepinus* fed dietary *Persea amaericana* and exposed to *Klebsiell, pneumonae* for seven (7) days. (53) also observed the presence of vacuoles in the liver of *P. aeruginosa* infected Common carp (*Cyprinus carpio*) exposed to chitosan and ciprofloxacin. The absence of vacuoles in the liver of the fish in groups D^{-ve} and D1 (Plate 1A and 1C respectively) could be a threat sign to the liver of the fish as a result of the presence of the *P. aeruginosa*. Other authors have reported nutritional deficiency, unfriendly environment and diseases presence as causes of absence of vacuoles in the liver of fish (16, 54, 55).

However the liver of the fish fed D1, which contains the lowest concentration of *I. batatas* had a mild infiltration by lymphocytes. This result supports the findings of (56) who reported an inflammation in the liver of *C. gariepinus* infected with *Aeromonas hydrophila*. The appearance of this mild infiltration could be as a result of the insufficient amount of *I. batatas* leaf extracts in diet D1 (50ml/kg) since fish fed with D2 (100ml/kg) and D3 (150ml/kg) had no infiltrations.

The medicinal phytochemicals in *Ipomoea batatas* leaf extracts such as flavonoids and alkaloids (19) prevented the experimental pathogen *P. aeruginosa* from affecting the liver of

the fish in groups D2 and D3, (16) stated that the presence of glycogen vacuoles depicts high energy intake and use, indicating a healthy liver.

6. CONCLUSION

The results of this study have successfully revealed that dietary supplementation within. *batatas* leaves aqueous extracts has a positive impact on the health of *C. gariepinus*, especially when they are challenged with *P. aeruginosa*pathogenic bacteria. This is likely due to the antioxidant and anti-inflammatory properties of the plant extract, which may help to protect the fish from oxidative stress and tissue damage caused by the pathogen. Additionally, the extract may also help to boost the fish immune system, fortifying them to better fight off infection. The inclusion of the herb (*I. batatas*) (50 – 150ml/kg) in the experimental diets did not affect the water quality of the experimental tanks. But precursion should be taken during the feeding of fish to avoid uneaten feed deposit as this may affect water quality.

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