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

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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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* challenged 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 ($D0^{+v}$ and $D0^{-ve}$). At the end of feeding period, the fish fed $D0^{-ve}$ (negative control) and D1-D3 were infected with 1.5ml of $1.5 \times 10^{10}_{cfu}$/ml overnight grown *Pseudomonas aeruginosa* while the fish fed $D0^{+ve}$ was unfected.

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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 while 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. 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 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. Sequel to these results, it is evidential that *I. batatas* possess the potentials of maintaining normal biochemical activities, and safeguard fish liver against *Pseudomonas aeruginosa*. It is therefore recommended that aquaculturist should embrace and apply this great discovery to maintain fish health especially in harsh environment.

Keywords: Pseudomonas aeruginosa; histopathological analysis; fish fed; animal protein.

1. INTRODUCTION

Fish and fish products are relatively cheaper compared to other sources of animal protein like beef and pork (Amao et al. 2006). 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 (Otene et al. 2018), and there is the need to culture fish outside it's natural environment. *Clarias gariepinus* is the most cultured fish species in Africa, and it is considered most suitable to culture outside its natural environment (Adewunmi and Olaleye 2011).

However, one of the major challenges facing fish farmers is the occurrence of diseases (Ukwe et al. 2019) due to the presence of virus, bacteria, parasites, fungi and some anthropogenic activities leading to an unhealthy and stressful environment that causes the suppression of the immune system and increasing the susceptibility of fish to infectious diseases (Awe et al. 2019, Awe and Deekae 2022). Fish diseases tend to spread quickly through the water and this is a source of great concern to those in the practice of aquaculture (Noraini et al. 2022). One of the infectious bacteria that causes diseases in African catfish is *Pseudomonas aeruginosa* (Ukwe and Jamabo 2020).

Fish disease causes economic losses and threat to consumers health. 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 (Hoai 2020). 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 (Rico et al. 2013) but many nations have restricted the use of these synthetic drugs because they possess serious risks on the environment and humans such as antibiotics accumulation in the tissue of fish, and significantly increasing pathogen resistance, and polluting the environment. (11, 12, 13). Herbs and herbal products have proven to be good solutions and 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, (Ukwe et al. 2019).

The presence of diseases and their causative pathogens can be detected in fish in various ways including: analysis of biochemical parameters (Gabriel et al. 2015, Ukwe 2021), histopathological and haematological assessment (Ukwe et al. 2021, Ukwe and Deekae 2022).

Ipomoea batatas (sweet potato) leaf possess significant nutrients and phytochemicals that can enhance productivity in aquaculture (Ukwe and Deekae 2024). The purpose of this research is to access the prophylactic properties of *I. batatas* in the development of aquaculture.

2. MATERIALS AND METHODS

2.1 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.

2.2 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 were 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.

2.3 Determination of Water Quality Parameters

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

2.4 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.

2.5 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 Ukwe and Jamabo (2020). *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.

2.6 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 Do, D1, D2 and D3 respectively.

2.7 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.

2.8 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, D2 and D3. At the end of the feeding period, the fish fed Do^{-ve} and D1 – D3 were infected intraperitoneally with 1.5ml of 1.5 x 10^{10}_{cfu}/ml overnight grown *pseudomonas aeruginosa*, while the fish fed Do^{+v} were uninfected, and were 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 histopathological analysis, while water quality was assessed before and after water exchange daily.

2.9 Blood Extraction

The fish was blind folded by covering the head with a thick cloth to attain calmness (Ukwe and Vopnu 2018) and blood was extracted via kidney puncture through the genital opening using 5ml injection syringe.

2.10 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.

2.11 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)

2.12 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 (Ukwe et al. 2023).

2.13 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.

2.14 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 (Wahua 1999).

3. RESULTS

3.1 Physicochemical Parameters of the Experimental Waters

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

3.2 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 *Pseudomonas 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 *Pseudomonas aeruginosa* (Mean ±SD)

Experimental Diets	Parameters				
	AST(IU/L)	**ALT(lU/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

Table 3. Metabolites activities in the plasma biochemistry experimental fish after seven days of exposure to *Pseudomonas aeruginosa* (Mean ±SD)

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;

Table 4. Electrolytes activities in the plasma biochemistry experimental fish after seven days of exposure to *Pseudomonas 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

3.3 Metabolite Activities in the Plasma Biochemistry of the Experimental Fish after Seven (7) days of Exposure to *Pseudomonas aeruginosa*

The values for Metabolite activities in the plasma biochemistry of the experimental *C. gariapinus* after 7 days of exposure to *Pseudomonas 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).

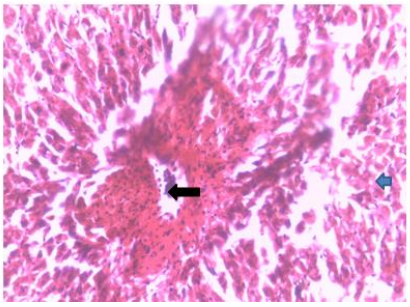
3.4 Electrolytes Activities in the Plasma Biochemistry of the Experimental Fish after Seven (7) days of Exposure to *Pseudomonas aeruginosa*

The values of Electrolyte activities in the plasma biochemistry of the experimental *C. gariapinus* after 7 days of exposure to *Pseudomonas 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.

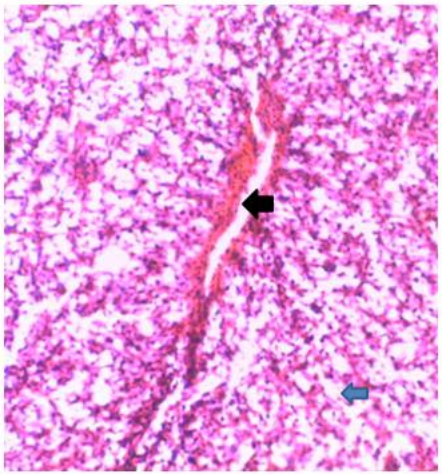
3.5 Liver Histopathology of the Experimental Fish after Seven (7) days of Exposure to *Pseudomonas aeruginosa*

The liver histopathology of the Experimental Fish after Seven (7) days of Exposure to *Pseudomonas aeruginosa* are shown in plates A – E.

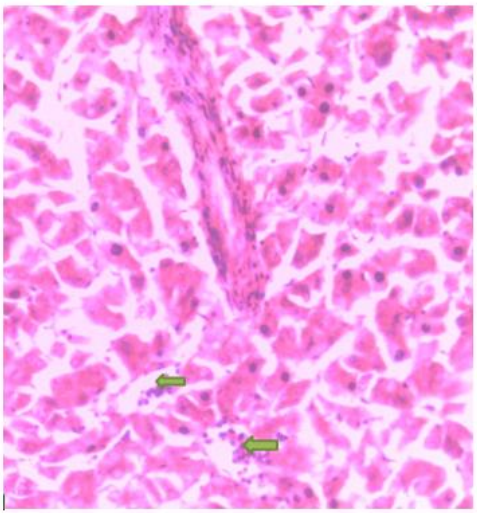
Plate A. is the liver of the fish from group D^{-ve}, it has normal hepatocytes (blue) and normal central vein (black) × 200. B is the liver of the fish in group D^{+ve} without infection, it has normal appearing glycogen vacuoles (blue) and normal central vein (blue) ×200. Plate C is the liver of the 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). Plate 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 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.



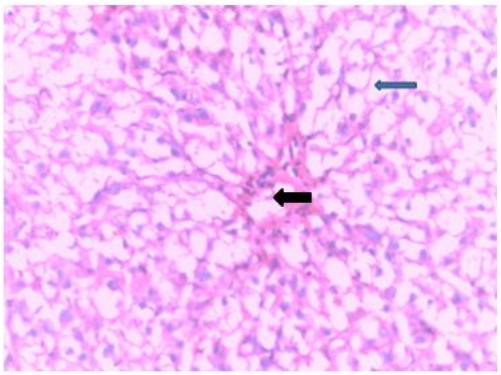
A. Section shows normal appearing hepatocytes (blue) and normal central vein (black) X200



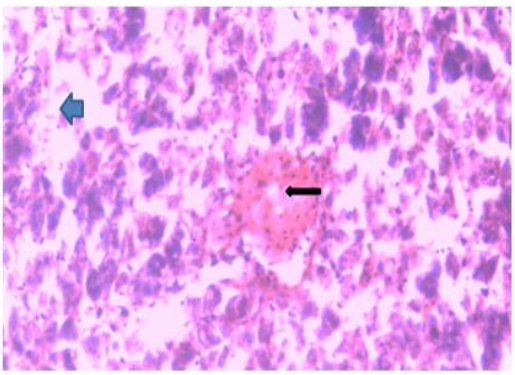
B. Section shows normal appearing liver containing glycogen vacuoles (blue) which is suggestive of a cultured fish and normal central vein (black) X200



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



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



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

4. DISCUSSION

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 4) supports the ultimate performance of fish (Ukwe and Deekae 2024, Ukwe and Abu 2016). 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 immunity against pathogens (Dineshkumar et al. 2014). 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 (Ukwe 2021, Dineshkumar et al. 2014, Rashannab et al. 2006), and increase in these enzymes suggests the presence of disease (Ukwe et al. 2019). 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 *Pseudomonas aeruginosa*. This result is in agreement with the findings of Ukwe et al. (2023) 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. Abu and Gabriel (2023) 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 (Ukwe et al. 2023, Abu and Gabriel 2023). However, the reduced activities of these enzymes (AST, ALT and ALP) in the Plasma of fish in group D1-D3 is 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 (Ukwe and Deekae 2024). Ukwe and Deekae (2022) reported that these phytochemicals have the capacity to boost the immune system of *C. gariepinus* against *Klebsiella pneumonia*, and (Ukwe and Deekae 2024) 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 (Alhassan et al. 2022). According to Clayton (2009), 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 Ukwe et al. (2023) who recorded increase in TB

and CB when *P. aeruginosa* infected *C. gariepinus* was exposed to varying concentrations of *Zea Mays* (corn) husk extracts. Hastuti et al. (2019) 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 (Ukwe et al. 2023), and increase in the CB level can be attributed to an obstruction in the bile duct (Nwamba et al. 2006). 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 *Ipomoea batatas* leaf extracts against *P. aeruginosa* (Suhendy et al. 2023, Ukwe and Deekae 2024).

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 (Ukwe et al. 2023), and alterations in these metabolites in fish blood can suggest innate immune response or disease presence (Ukwe et al. 2023, Bahmani et al. 2001). 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 and D^{+ve} after seven days of exposure to *Pseudomonas aeruginosa*. This result supports the result obtained by Ukwe et al. 2023 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 Pawar et al. 2024 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 (Muhmoud and Abasals 2010) 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 (Ukwe and Deekae 2024). Osuntokun et al. 2020 had earlier reported antimicrobial activities of *I. batatas* leaves extract against *P. aerguniosa*.

Urea is a major product of the breakdown of proteins in the body Adam et al. (2017) while creatinine is a major pointer to kidney functionality (National Kidney Foundation 2002). 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 Ukwe et al. (2023) 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 kidney of the experimental fish (Nnabuchi et al. 2015). 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 (Ukwe and Deekae 2024, Reyna and Subha 2024), and it reveals the fact that dietary *I. batatas* leaves protected the kidney against *P. aeruginosa.*

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 (Fazio et al. 2021) because they have osmoregulatory functions (Shui et al. 2018). 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 Koeypudsa et al. (2020) 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 (Hassan et al. 2023, Gopal et al. 1997).

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 Wilfred–Ekpr (2021) 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. Iheanacho et al. (2022) 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 (Edori et

al. 2013). 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 Obum-Nnadi (2022) 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 Gabriel et al. (2012) who reported the alterations of selected electrolytes in organs of *C. gariepinus*. The similarities of HC0 $HC\,0_3^-$ in all groups suggests that the experimental pathogen *P. aeruginosa* did not alter the respiratory system of the fish.

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 targeted organs such as gills, liver, spleen etc (Oghenoclhuko et al. 2021). After seven (7) days exposure to *P. aeruginosa*, the fish in groups D2 (Plate D) and D3 (Plate E) had normal appearing glycogen vacuoles (blue) and normal central vein (black) in their liver, similar observation was seen in group D^{+ve} (Plate B). These features are suggestive of a cultured fish (Ukwe et al. 2021). Whereas the liver of the fish in groups D^{-ve} (Plate A) and D1 (Plate C) had appearing hepatocytes and normal central vein (black) and no glycogen vacuoles. This results is in consonant with the report of (Ukwe et al. 2021) 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. Al-Mossawai et al. (2019) 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 A and C 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 (Ukwe et al. 2021, Vallejor et al. 1998, Dridi et al. 2015).

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 Mufidah et al. (2022) 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 (Ukwe et al. 2023) prevented the experimental pathogen *P. aeruginosa* from affecting the liver of the fish in groups D2 and D3 (Ukwe et al. 2021) reported that the presence of glycogen vacuoles depicts high energy intake and use, indicating a healthy liver.

5. CONCLUSION

The results of this study has successfully revealed that dietary supplementation with *Ipomoea batatas* leaves aqueous extracts has a positive impact on the health of *Clarias gariepinus*, especially when they are challenged with pathogens such as *Pseudomonas aeruginosa*. This is likely due to the anti-bacterial and anti-inflammatory properties of the plant extract, protecting the fish from microbial attacks and tissue damage caused by pathogens such as *P. aeruginosa*. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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