Acta Scientific AGRICULTURE (ISSN: 2581-365X)

Volume 8 Issue 12 December 2024

Review Article

Pharmacological Activities and Hepatoprotective Ability of Soursop (*Annona muricana*) Powdered Leaves on Clarias Gariepinus Infected with *Klebsiella Pneumoniae*

Ukwe IOK[1]*, Edun OM[2] and Oladapo-Akinfolarin TT[3]

[1]*Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University, Nigeria*

[2]*African Regional Aquaculture Center/Nigeria Institute of Oceanography and Marine Research, Port Harcourt, Rivers State, Nigeria*

[3]*Department of Clinical Chemistry, Faculty of Medical Laboratory Science, Rivers State University, Nigeria.*

***Corresponding Author:** Ukwe IOK, Department of Fisheries and Aquatic Environment, Faculty of Agriculture, Rivers State University, Nigeria.

Received: November 05, 2024

Published: November 26, 2024

Abstract

Phytochemical screening of powdered *A. muricana* leaves was conducted to evaluate its pharmacological activities. Varying quantities of the *A. muricana* leaves were added to locally formulated 40%cp feed to produce five different diet as follows: 0g/kg, (control) 5g/kg, 10g/kg, 15g/kg and 20g/kg of A. muricana /kg of feed, and labeled as A, B, C, D and E respectively. 150 sub-adult Clarias gariepinus were divided into 5 groups (A-E) in triplicates of 10 fish/replicate using fifteen 50L plastic aquaria, and feeding with the various diets commenced after 24 hours. After eight (8) weeks of feeding with the experimental diets (A-E), the fish were infected with 1.0ml of 3.8 x 108 cfu/ml *Klabsiella pneumonae*, and observed for seven (7) days. Blood samples were collected from each groups (A-E) after eight days feeding, and seven days post infection period to determine the activities of aspartate aminotransferase (AST), alanine aminotransferace (ALT) and alkaline phosphate (ALP), and the total bilirubin (TB) and conjugated bilirubin (CB) of the fish plasma, while liver was harvested from the fish in the various groups after 7 days post infection period to determine the histopathology and bacteriology of the liver. The results reveals that after eight weeks feeding the *A. muricana* enhanced liver health by reducing the quantity of liver enzymes AST and ALT discharge to the fish plasma as lower values were recorded in diets B-E compared to diet A. After the seven days infection, activities of the AST, ALT and the TB and CB content was significantly higher in the fish fed the control diet A compared to the fish fed dietary A. muricana (B-E), while the histopathological analysis reveals grade 2 steatosis with severe hepartocytes in fish fed diets A and B, grade 1 steatosis in fish fed diets C, while fish fed diets D and E had no steatosis but lymphocytes infiltration was observed in the liver of fish fed diet E. The liver of the fish fed diet A (control) was highly contaminated with K. pneurnonia compared to the liver of the fish fed diets B-D with significantly lower concentration of *K. pneurnonia*. The phytochemical evaluation of *A. muricana* reveal hudge pharmacological activities such as anti-bacterial, anti-cancer, hepatoprotective etc. The results of this research shows that *A. muricana* powdered leave exhibited series of pharmacological activities and hepatoprotective effects in *C. gariepinus* even in the presence of bacterial pathogen.

Keywords: Clarias Griepinus; *K Pneumonae; Annana Muricana*; Pharmacological Activities; Hepatoprotective ability

Introduction

The high demand for fish and fish products is causing serious decline in the presence of fish in the wild. Fish is the cheapest source of animal protein in underdeveloping and developing countries in the world. Aquaculture accounts for over fifty (50) percent of fish production all over the world and remains the fastest growing food production sector. Nigeria is among the largest consumers of fish in the world, and the highest in Africa [1], with Tilapia and African Catfish as the most accepted and cultured spaces [2]. Lack of fish seeds, feed and disease presence are the major problems facing the aquaculture industries, with disease presence taking the lead. Disease in fish is caused by one or more of the following: (i) invasion of the environment with pathogens (bacteria, viruses, fungi and parasites); (ii) weak immune system of the fish and (iii) unsatisfactory water environment (dissolve oxygen, temperature and pH) that leads to stress. Fish in aquaculture is exposed to infection in so many ways, most especially invading pathogen and polluted environment [3,4].

Haemato-biochemical indices are known tools in monitoring the health status of fish with respect to disease presence or infection [3,5], since alterations in these indices depicts diseases presence or stress from poor environment, contamination and in some cases poor pond management [5]. further stated that different pathogen alters the biochemical component of the fish plasma differently. Liver enzymes such as aspartate amino transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) are said to be indicators of fish infections, harsh environment and tissue damage especially the liver of fish [7-9] attributes increase in ALP in fish fed dietary herbs to enhanced immune system. Total bilirubin (TB) and conjugated bilirubin (CB) content of the blood are used as assessors for the proper functioning of the hematopoietic organs such as liver, spleen and kidney [10,11] agreed that the AST and ALT are observed in high quantities in fish tissues when injuries or diseases affects the said tissues, destroying the tissue cells and releasing AST and ALT into the plasma. Phytochemicals are known to reduce the negative effects of infection and diseases in fish by the activitation of the fish immune system or been bactericidal to the causative agent [12-14]. The importance of phytochemicals have also been reported in aquaculture in areas such as growth and fish health management [15]. *Annona muricana* have been reported to possess some medicinal phytochemicals that enhance growth and maintain health in animals [16].

The purpose of this research work was to evaluate the hepatoprotective effect of *A.muricana* powdered leaves on the liver of *C. gariepinus* before and after exposure to *Klebsiella pneumonae.*

Materials and Methods

Experimental area

The study was carried out in the Department of Fisheries and Aquatic Environment, Rivers State University, Nigeria.

Purchasing and acclimatization of fish

One hundred and fifty (150) sub-adult *Clarias gariepinus* was purchased in the fish farm and acclimatized for two weeks, while observing disease presence/bruises. During the period of acclimatization the fish was fed to satiation with commercial diets.

Processing of Soursop (A. muricana) leaves powder

Annona muricana was collected within Port Harcourt in Rivers State, and processed using the method of [17]. The leaves were washed clean and air dried for two (2) weeks. The dried leaves were ground to powder using locally fabricated machine and was sieved using a plastic mesh held in a frame to remove chaff. The fine powder attained was put in a plastic can and stored in the refrigerator for use.

Quantitative screening of *A. Muricana* powdered leaves

The quantitative screening of the leaves powder to ascertain its phytochemical content was carried out in the Department of Biochemistry, Faculty of Science, Rivers State University in accordance with International Organization for Standardization (ISO) 179245 using 250 visible.

Experimental diets

Forty percent (40%) crude protein feed was formulated with local ingredients (Bloodmeal, fishmeal, groundnut cake, *Vigna subterrenae,* corn, wheat offal, vitamin C, fish preanix, lysine, methionine, palm oil, and starch) using Pearson square method. Five different diets were prepared from the formulated feed using varying quantities of the prepared *A. muricana* powder leaves per kilogram

of the feed as follows: Og/Kg; 5g/kg; 10g/kg; 15g/kg and 20g/kg. The various diets were properly mixed and extruded in sizes of 6mm and 9mm with extruding machine to produce floating feeds. The feeds were sundried, bagged in an air-tight bag and labeled; A (0g/kg), B (5g/kg), C (10g/kg), D (!5g/kg) and E (20g/kg), and stored in the laboratory.

Experimental pathogen (*K. pneumonae*)

A pure colony of *Klebsiella pneumoniae* was cultured overnight in nutrient broth at 37°C in an aerobic incubator for 24 hours. The concentration of the *K. pneumonia* was determined by serial dilution of the overnight broth in normal saline up to 8 fold dilution (10^8). The last dilution was cultured on nutrient agar and incubated aerobically at 37°C for 24 hours in an aerobic incubator for enumeration. The final concentration in the stock solution was $3.8x10^8$ *cfu*/ml.

Feeding of fish and infection with *K. Pneumonia*

After this acclimatization, the 150 sub-adult *Clarias gariepinus* were distributed into five groups in triplicate of ten using fifteen 50L plastic aquaria. Each of the five groups were fed diets A – E accordingly. Feeding started 24 hours after stocking. After eight (8) weeks of feeding with the experimental diets, blood samples were collected from the fish fed A – E for enzymes and bilirubin analysis, and the fish were interperetoneally infected with 1.0ml of 3.8 x 10^8*cuf*/ml *K. pneumonia* after 6 hours, and feeding with the diets continued. After seven days post infection period, blood sample were again collected from each of the replicate and taken to the laboratory for enzymes and bilirubin analysis. Livers from the fish in each group were harvested and taken to the laboratory for histopathiological and bacteriological analysis.

Blood extraction

The head of the fish was properly covered with a thick cloth to attain calmness and blood samples were collected via kidney puncture through the genetal opening [18] using injection syringe and niddle, transferred into a lithum heparin tube and taken to the laboratory within 6 hours.

Enxyme and bilirubin analysis

The blood was assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TP) and conjugated bilirubin (CB). It was done by the use of "Evolution 300 machine" an auto-analyzer, screen master model. Manufactured by Biochemcial system, England. It was used according to manufacturers instructions.

Histopathological analysis

The harvested fish livers were immersed in 10% formalin solution in five different sample bottles and taken to the histopathology laboratory in the University of Port Harcourt, Rivers State, Nigeria. The liver samples were processed and trimmed using a rotary microtone (LEICA RM 2125 RTS), manufactured by LEKA Brosysteo, Buffalo Groce 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 x10 magnification.

Bacteriological analysis of fish livers

One (1) gram of each of the liver was separately homogenized and suspended in 10ml of normal saline serially diluted. The diluted solutions were cultured on a MacConkey agar plate and incubated at 37°C in an aerobic incubator, and viable colonies were counted after 16 hours of incubation.

Water parameters

Temperature, pH and dissolved oxygen (Do) were analyzed before and after every water exchange using Mecury-in-glass thermometer, pH meter (model, PHS- 25, Techmel and Techmel USA), and D0. – meter (BANTE 980 Precision version: 2009070200 manufactured by BANTE INSTRUMENTS Co- LTD, China) respectively.

Statistical analysis

The obtained data were subjected to descriptive statistics and one way analysis of variance (ANOVA) and Duncan's multiple range test was used to separate means at significant level of $P<0.05$ (Duncan, 1966) using 18m statistical package (SPSS) version 22.

Results

Quantitative Screening of *A. muricana* Leaves

The result of the plytochemical screening is shown in Table 1. It reveals the significant presence of flavonoids, saponnins, alkaloids and phenols, and less presence of tannis, quinones, triterpenoids and carotenoids. These observation suggests that *A. muricana* leaves possess some pharmacological activities.

Physicochemical Parameters of the experimental waters

The result for the physicochemical parameters of the experimental waters are shown in table 2. The values of all the evaluated water parameters were similar across the various diets A-E. Dissolve oxygen: 5.13 ± 0.19-5.53 ± 0.17; pH: 6.59 ± 1.2- 6.85 ± 0.12 and Temperature: 25.99 ± 2.1 - 27.01 ± 12.

Enzymes Activities and Bilirubin content of *C. gariepinus* after

eight (8) weeks feeding with dietary *A. muricana*.

The result of the above is shown in table 3. The AST was higher in fish fed diet A (control) but similar and lower in fish fed diets B-E, while the ALT was similar and higher in diets A, B, and C but similar and lower in diets D and E. However, the value of the ALP was significantly higher in the fish fed diets E (25g/kg of *A. muricana*/feed), but lower and similar in other diets (A-D). The result of the TB and CB were similar across all the experimental diets (A-E).

Enzymes Activities and Bilirubin content of the experimental fish after seven (7) days of *K. pneumonae* Infection

The result of the seven (7) days post infection period with *K. pneumonae* is shown in table 4. The AST and ALT were significantly higher in the fish fed diet A (control diet), but similar and lower in the fish fed varying quantities of *A. muricana* (B-E), but the ALP was significantly higher in diets E, but similar in diets A-D. The results of the TB and CB showed significantly higher values in fish fed diet A, compared to the significantly lower and similar values in diets B-E.

The Bacteriological Analysis of *K. pneumonae* in the liver of *C.*

S/N	Components	Quantity
1	Flavonoid	35.61 ± 0.05
2	Coumarins	-
3	Saponins	44.61 ± 0.01
4	Tannins	0.37 ± 0.00
5	Quinones	1.13 ± 0.01
6	Alkaloids	39.08 ± 0.09
7	Triterpeniods	0.34 ± 00 (NC)
8	Proanthyocyanidin	-
9	Phenol	20.68 ± 0.00
10	Steroids	-
11	Carotenoids	2.03 ± 0.01

Table 1: Quantitative Screening for Phytochemical Components in *A. muricana* Powdered Leaves.

	Diets				
Parameters	**A**	**B**	**C**	**D**	**E**
D0(Mg/L)	5.53 ± 0.17^{a}	5.51 ± 0.41^{a}	5.13 ± 0.19^{a}	5.21 ± 0.14^{a}	5.29 ± 0.61^{a}
pH	6.8 ± 0.31^{a}	6.85 ± 0.12^{a}	6.81 ± 0.31^{a}	6.59 ± 1.2^{a}	6.79 ± 0.51^{a}
Temperature (°C)	26.1 ± 0.31^{a}	25.99 ± 2.1^{a}	26.3 ± 15^{a}	27.01 ± 12^{a}	26.99 ± 0.13^{a}

Table 2: Physiochemical Parameters of the Experimental Waters within the Period of the Experiment (Mean + SE).

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

Key: Do: Dissolve oxygen; Temp. Temperature, pH; hydrogen ion concentration.

Parameters	Experimental Diets (Mean ± SD)				
	A	**B**	**C**	**D**	**E**
AST	10.67 ± 7.37^{a}	6.33 ± 1.53^{b}	6.67 ± 0.58^{b}	6.00 ± 1.00^{b}	7.67 ± 0.58^{a}
ALT	10.00 ± 1.00^{a}	11.00 ± 4.58^{a}	10.33 ± 3.21^{a}	7.33 ± 1.53^{b}	8.00 ± 1.73^{b}
ALP	47.33 ± 3.21^{b}	47.33 ± 3.79^{b}	47.67 ± 0.58^{b}	46.00 ± 6.00^{b}	53.67 ± 23.18^{a}
TB	2.03 ± 1.45^{a}	2.20 ± 0.10^{a}	2.20 ± 0.17^{a}	2.03 ± 0.15^{a}	2.33 ± 0.15^{a}
CB	1.80 ± 0.87^{a}	1.27 ± 0.06^{a}	1.37 ± 0.12^{a}	1.23 ± 0.15^{a}	1.37 ± 0.25^{a}

Table 3: Enzyme Activities and Bilirubin content in plasma Biochemistry of *C. gariepinus* fed Dietary *A. muricata* for eight (8) weeks.

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

Key: AST (IU/l): Aspartate Aminotransferase; ALT (IU/l): Alanine Transaminase; ALP (IU/l): Alkaline Phosphatase; TB(mmol/L): Total Bilirubin; CB (mmol/L): Conjugated Bilirubin

Parameter	Experimental Diets (Mean ± SD)				
	A	B	C	D	E
AST	16.00 ± 4.00[a]	12.33 ± 5.86[b]	10.33 ± 7.09[b]	10.00 ± 2.00[b]	9.33 ± 7.57[c]
ALT	18.67 ± 3.06[a]	9.67 ± 1.53[c]	10.00 ± 2.00[b]	10.33 ± 1.15[a]	10.33 ± 2.52[a]
ALP	41.67 ± 6.51[c]	45.33 ± 2.08[b]	43.33 ± 1.53[c]	42.33 ± 4.04[c]	48.00 ± 8.19[a]
TB	6.63 ± 1.90[a]	2.90 ± 1.22[c]	3.13 ± 0.91[b]	2.73 ± 0.68[c]	2.67 ± 0.38[c]
CB	5.07 ± 1.12[a]	2.30 ± 0.26[b]	2.23 ± 0.81[b]	1.80 ± 0.46[c]	1.90 ± 0.26[c]

Table 4: Enzyme Activities and Bilirubin content in plasma Biochemistry of *C. gariepinus* fed Dietary *A. muricata* for eight (8) weeks and infected with *K. pneumonae* for seven (7) Days Means within the same row with different superscripts are significantly different ($P < 0.05$)

Key: AST (IU/l): Aspartate Aminotransferase; ALT (IU/l): Alanine Transaminase; ALP (IU/l):Alkaline Phosphatase; TB(mmol/L): Total Bilirubin; CB (mmol/L): Conjugated Bilirubin

gariepinus fed dietary *A. muricana* after seven (7) days infection.

The result of the bacteriological analysis is shown in Table 5. The liver of the fish fed diet A had significantly higher content of *K. pneumonae (3.*65 ± 0.070 x 10^7 *cfu/ml*), while the fish fed with the various quantity of *A. muricana* diets (B-E) had similar results of less than 0.005 x 10^7 (fu/ml) in their liver.

fish After seven (7) days infection with *K. pneumonae*.

The result of the histopathological assessment of the fish fed diets A-E is shown in plates A-E respectively. The liver tissues of plates A and B had grade 2 steatosis, while plates C had grade 1 steatosis. Plates D and E had no steatosis but plate E had serious lymphocytes infiltration.

SECTIONS OF LIVER TISSUE SHOW NORMAL CENTRAL VEIN

Histopathological Assessment of the liver of the experimental

Treatments	Experimental Fish Organ Liver ($x10^7_{cfu}$/ml)
A	3.650 ± 0.070[a]
B	0.003 ± 0.001[a]
C	0.001 ± 0.0001[b]
D	0.002 ± 0.0001[b]
E	0.001 ± 0.0001[b]

Table 5: Bacteriological Analysis of *K. preumonae* in the Liver of *C. gariepinus* Fed Dietary *A. muricana* after Seven (7) Days Infection. Mean within the same row with different superscript are significantly different ($P < 0.05$).

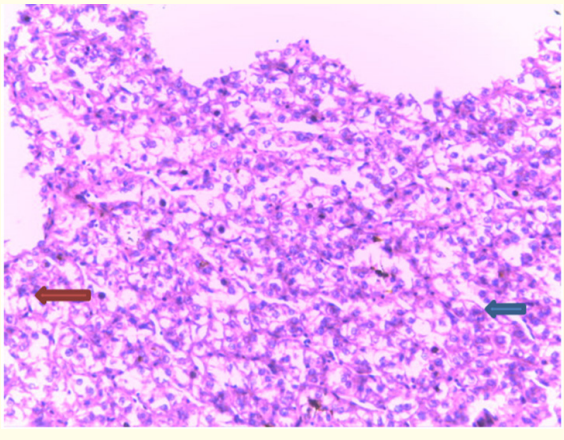

Figure A: (0g/kg).

(RED) AND ABOUT 50% OF HEPATOCYTES FILLED WITH MICRO AND MACROVESICLES CONTAINING LIPID (BLUE) SUGGESTIVE OF GRADE 2 STEATOSIS.

SECTIONS OF LIVER TISSUE SHOW CENTRAL VEINS CONTAINING NUMEROUS LYMPHOCYTES (RED) AND ABOUT 50% OF HEPATOCYTES FILLED WITH MICRO AND MACROVESICLES CONTAINING LIPID (BLUE) SUGGESTIVE OF GRADE 2 STEATOSIS.

SECTIONS OF LIVER TISSUE SHOW CENTRAL VEINS CON-

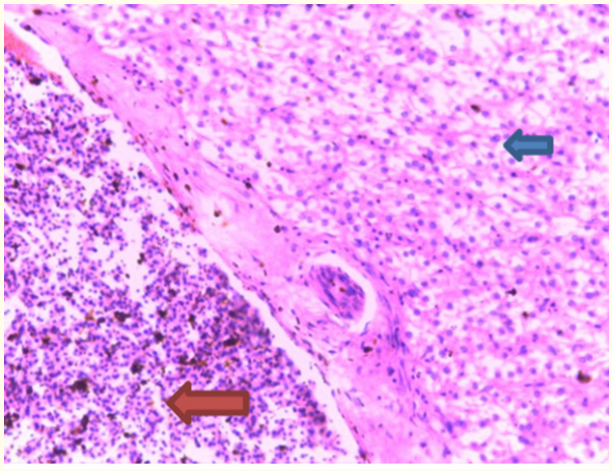

Figure B: (5g/kg).

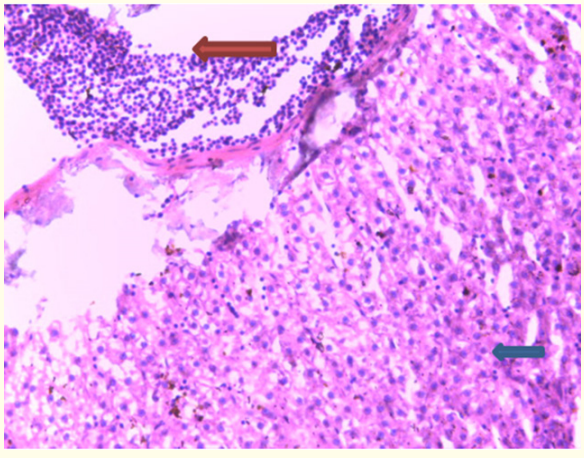

Figure C: (10g/kg).

TAINING NUMEROUS LYMPHOCYTES (RED) AND ABOUT 10% OF HEPATOCYTES FILLED WITH MICRO AND MACROVESICLES CONTAINING LIPID (BLUE) SUGGESTIVE OF GRADE 1 STEATOSIS

SECTIONS OF LIVER TISSUE SHOW CENTRAL VEINS CONTAINING NUMEROUS LYMPHOCYTES (RED) AND NORMAL HEPATOCYTES (BLUE)

SECTION SHOWS A FOCUS OF NECROSIS OF HEPATOCYTES

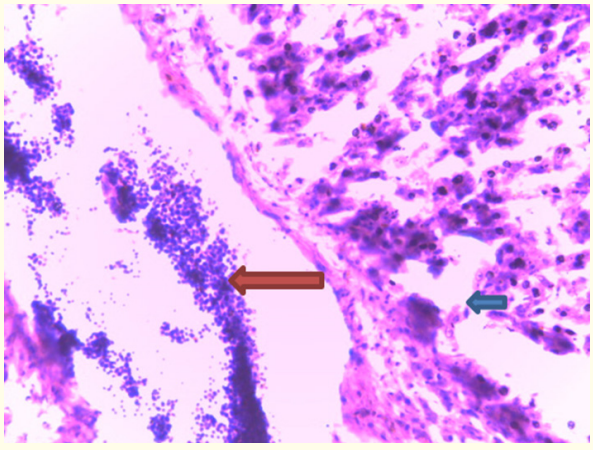

Figure D: (15g/kg).

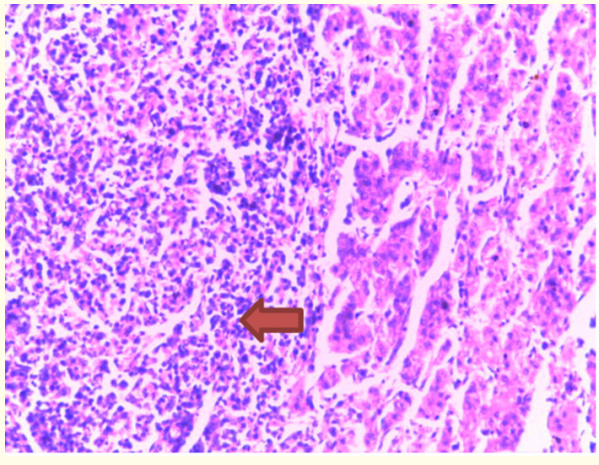

Figure E: (20g/kg).

(RED) AND HEAVY INFILTRATION BY LYMPHOCYTES WITHIN SUCH FOCI.

Key of Steatosis

- GRADE 0- LESS THAN 5%
- GRADE 1- 5-33%
- GRADE 2- 34-66%
- GRADE 3- GREATER THAN 66%

Discussion

Pharmacological Activities of *A. muricana* Powdered Leaves

Plants are sources of phytocehmicals with enormous pharamacological activities [19]. The phytochemical screening of *A. muricana* leaves reveals the presence of the following phytochemicals in various quantities: flavonoids, saponnins, tannins, quinones, alkaloids, triterpenoids, phenols and carotenoids (Table 1). Similar result was reported by [16] in the evaluation of *A. muricana* leaves powder for its phytochemicals and proximate composition. These phytochemicals displays series of pharmacological and bioactive activities in animals when consumed as food or drugs.

Flavonoids and phenols have so many pharmacological activities such as antioxidant enhancement, cardioprotective potential, antibacterial, anti-cancer, anti-inflammatory, hepatoprotective etc [20-22]. Flavonoids demonstrates antioxidant potentials against oxidative stress by activating antioxidant enzymes [23,24], and demonstrates anti-inflammatory activities by scavenging reactive oxygen species and boosting the immune system [22]. further

stated that flavonoids demonstrates hepatoprotective effect by reducing the levels of aspartate and alanine amminotransferace (AST and ALT) in the plasma. Oxidative stress that leads to many diseases such as cancer, cardiovascular disease, neurologic malfunction, inflammatory disease etc are produced by the presence of oxygen radicals referred to as reactive oxygen species [25,26]. which are eradicated from the plasma by phenols and flavonoids [16,27]. Alkaloids are plants phytochemicals with series of pharmacological activities as therapeutant [28,29]. Alkaloids have demonstrated therapeutic tendencies as inflammatory mediators, mediators of neurodegenerative disorders, antioxidants etc [25] and account for 60% of plant derivative drugs [30,31] stated that alkaloids exhibit some pharmacological activities such as antibacterial, antiviral, antiproliferation and insecticidal due to the availability of protons receiving N-atoms and protons donating amine H – atoms. The presence of saponins in plants leaves have been reported by so many authors [14,16,34] and factors such as genetic origin and age of the plant determines the content and composition of saponins [33,34]. reported saponins to be anti-cancer and sugar reducing agents. Saponin have also been reported to be anti-microbial, anti-inflammatory and anti-viral etc [35,36]. Carotenoids have been reported to prevent cancer and heart diseases [37,38]. reported the antioxidant and antimicrobial activities of quinones when the bark extracts of *Rhamnus alaternaus* and *Rhamnus fallax* were analysed, while [16]. reported the usefulness of tannins in the prevention of cancer and treatment of ulcers.

Physicochemical parameter of the experimental water

The nature of the culture water determines the success of aquaculture [39]. The result of the physicochemical parameters in this work are similar to the one reported by [39] and are acceptable for a productive aquaculture practice [40]. This result implies that the experimental diets A-E have no negative effects on the physicochemical parameters of the water.

Hepatoprotective Effects of *A. muricana* Powdered leaves on *C. gariepinus* Before and After Infection with *K. pneomanae*

Activities of aspartate transaminase (AST), alamine transaminase (ALT) and alkaline phosphatase (ALP) which are known liver enzymes [10,41] are used as biomarkers to access the functionality of the liver [42]. The functionality of the fish liver to a large extend expresses the relationship between the fish health and the environmental agents especially in the aquatic ecosystem [43], because of its nutrient storage and detoxifying activities. Plasma bilirubin is a measure of the production and destruction of red blood cells, and the ability of the liver to conjugate the dead cells [10,44]. further stated that high levels of bilirubin in the blood indicates bile duct injury. Feed additives such as plants parts, environmental pollution and disease presence etc have been reported to alter liver functions of aquatic animals such as fish [45,46], and increase in the ALT, AST, ALP, total bilirubin (TB) and conjugated bilirubin (CB) have been attributed to infrindgement of the normal functioning of the liver [10,47].

The result of this research after eight (8) weeks feeding with the dietary *A. muricana* (Table 3) reveals that there were significant reduction in the AST and ALT activities in fish plasma fed dietary *A. muricana* (B-E) compared to fish fed control diet (A), but significantly reduced activities of ALT were recorded in the plasma of fish fed high levels of *A. muricana* (D and E). The reduced levels of ALT and AST in the fish fed dietary *A. muricana* (B-E) can be attributed to the protective tendency of the phytochemicals in *A. muricana* diets (B-E) on the liver, limiting the quantity of AST and ALT discharge to the plasma. *A. muricana* contains flavonoids reported to be hepatoprotective [22]. The activities of ALP was significantly higher in fish fed diet with the highest inclusion level of *A. muricana* (E) compared to the rest that were similar but higher in fish fed C and D. Higher activities of ALP have also been associated with enhanced immune system in fish [8]. The improved ALP activities in the plasma of fish fed dietary *A. muricana* depicts the immunoprotective properties of the *A. muricana* leaves on the liver of the experimental fish (*C. gariepinus*) (Rooster., *et al,* 2014). The TB and CB were similar in all the fish fed diets A-E indicating a balance in the production and destruction of red blood cells in the fish plasma, a situation that depicts normal conjugating and screening of bilirubin in the plasma [48], and this can be attributed to hepatoprotective capacity of the phytochemicals in *A. muricana*.

After seven days post infection with *K. pnumonae,* there were significant increase in the activities of AST, ALT, TB and CB values in the experimental fish fed the control diet (A) compared to fish fed diets B-E (Table 4), the increase in these parameters in the fish fed the control diet (A) depicts threat to the fish liver. Several authors have reported similar results: [46], when *Clarias gariepinus* was infected with *Pseudomonae aeruginosa* and [49] who reported increase in AST and ALT when *Oreochromus noliticus* and *C. gariepinus* were infected with external parasites. The increase in ALT and AST in the plasma of the fish fed control diet (A) after the infection could be as a result of cellular damage in the hematopoietic organs such as the liver, kidney etc [5,10,50]. Increase in the TB

and CB as seen in this work (Table 4) could be as a result of inability of the liver to conjugate excess bilirubin arising from the abnormal break down of red blood cells [51] and this can be attributed to the malfunctioning of the bile duct [10]. However, there were reduced values of these parameters (ALT, AST, TB and CB) in the plasma of the fish fed dietary *A. muricana* (B-E), and this can be attributed to phytochemicals such as flavonoids, alkaloids etc that have been found in *A. muricana* (Table 1) that are bacteriostatic or bactericidal to *K. pneumonae* [14,16]. These phytochemicals may also play the protective role on the liver by reducing the virulence of *K. pneumonae* [42]. The ALP had a different trend, it was significantly higher in the fish fed the diets (B-E) compared to the fish fed the control diets (A). The increase of the ALP in fish fed diets B-E depicts enhanced anti-microbial activities of the diets against the experimental pathogen (9) and this can be attributed to the phytochemicals present in the *A. muricana* powdered leaves [16].

The liver histopathology (Plates A-E) reveals different grades of hepatic steatosis. Hepatic steatosis is a situation were the liver fat is beyond five percent (5%) of the liver weight [52]. The presence of hepatic steatosis hinders the functions of the hepatocytes, facilitates damage of deoxyribonucleic acid (DNA) and alters the shape of the liver [53]. It is attributed to persistent bacterial infection which is often associated with lack of Vitamin D (54), but in some cases viable bacterial pathogen can cause liver diseases such as hepatic steatosis [55,56]. Hepatic steatosis have also been reported in fish fed high fat diet [57,58]. stated that hepatic steatosis is associated with inflammation and hepatocyte changes, and further categorized steatosis into three as follows: SI (< 10% hepatocytes); S2 (10-30% hepatocytes) and S3 (>30% hepatocytes) and describe them as mild, moderate and severe respectively. Though the liver has no bacteriostatic potentials, its products such as the bile have been reported to be bacteriostatic [59]. In this study the liver of the fish fed 0g/kg (A) and 5g/kg (B) had grade two steatosis (Plates A and B respectively), while the liver of the fish fed 10g/kg (C) (Plate C) had grade one steatosis. The presence of steatosis could be as a result of the *K. pneumonae* infection in the fish.

This assertion is supported by [60,61], both authors reported that high infection levels in the liver of fish negatively alters the performance of the liver. The different grades could be as a result of the varying quantities of *A. muricana* leaves powder in their diets, leading to enhanced functions of the liver as a fat emulsifier. However, the fish fed 15g/kg had no steatosis, normal lymphocytes and hepatocytes presence (Plate D) while the liver of the fish fed 20g/kg (Plate E) had no steatosis but necrosis of hepatocytes and infiltration by lymphocytes. The result in plate D depicts that the percentage of powdered *A. muricana* present in the feed was adequately bactericidal and possess no toxicity to the fish liver, while the result in Plate E suggests that the percentage inclusion of the *A. muricana* in the feed was high and despite been bactericidal it was toxic to the liver of the fish leading to leaching of the hepatocytes. The bactericidal activities of the *A. muricana* could be as a result of the presence of some of its phytochemicals, that have been reported to be bacteriostatic and bactericidal [14,39]. Some of these phytochemicals have also been reported to be toxic when in excess [62].

The results of the liver bacteriology (Table 5) reveals that the liver of the fish fed 0g/kg of feed (A) had significantly higher bacterial load compared to liver of the fish fed 5g/kg – 20g/kg (B-E). The significantly higher bacterial load on the liver of the fish fed A could be as a result of absence of bacteriostatic activity in the liver that may have aided the proliferation of the injected pathogen (*K. pneumonae)* [59]. The reduced bacterial load in the fish fed 5g/kg – 20g/Kg could be as a result of the antibacterial activities of the phytochemicals present in the *A. muricana* powdered leaves that are bacteriostatic or bactericidal to the injected pathogen (*K. pneumonia*). These phytochemicals have earlier been reported to be bactericidal to *K. pneumonae* and other bacterial pathogen [62,63].

Conclusion

Annona muricana powdered leaves have series of pharmacological activities. It exhibits hepatoprotective tendencies in aquatic organisms such as *C. gariepinus* especially in the presence of bacterial pathogens such as *K. pneumonia* and prevents liver steatosis in fish that may occur as a result of bacterial infection or fatty feeds. The best result was achieved in fish fed diets D and E. More research should be carried out with *A muricana* in areas of fish growth and fish flesh quality.

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