Phytochemcial Assessment of *Persea americana* Powdered Leaves and Its Potency in Protecting *Clarias gariepinus* against *Klebsiella pneumonia*

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1. ABSTRACT

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The phytochemcial content of *P. americana* powdered leaves was assessed using ethanol extracts. The potency of the powdered leaves was assessed by exposing *C. gariepinus* fed different levels of the dietary powdered leaves (Do: 0% leaves inclusion; D1: 3% leaves inclusion; D2: 6% leaves inclusion; D3: 9% leaves inclusion and D4: 12% leaves inclusion diets) for eight ~~(8)~~ weeks to *K. pneumonia* via intrapretoneal injection. The fish were injected after the 8 weeks feeding at day 1, 7 and 14. The haematological parameters and microbial load (antibacterial activities) of the infected fish blood and organs were assessed 21 days after the first injection. The ethanol extracts of the *P. americana* leaves powder revealeds~~s~~ the following phytochemicals: phenol, carotenoids, alkaloids, flavonoids, saponin, steroids, coumarine and proanthocyanidin in varying quantities. The haematological parameters in the infected fish compared to the control Do indicated that there were improved packed cell volume (PVC), Haemoglobin (Hb) and Red blood cells (RBC) in fish fed D2-D4 compared to the fish fed Do (control). The white blood cells (WBC), the lymphocytes, monocytes and Eosinophils were higher (P>0.05) in the fish fed Do compared to the fish fed D1-D4. The Red blood indices (MCH, MCHC and MCV) fluctuated in values across the diets, but were higher in fish fed D1. The microbial load was higher (P<0.05) in the organs (Kidney, spleen, liver and Blood) of the fish fed Do compared to the rest, and the effect was dose dependent. The results showeds~~s~~ that *P. americana* powdered leaves improved the haematological and antibacterial activities of the experimental fish.

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Keywords: Acquaculture, fish health, *Persea americana*, *Klebisiella pneumonia*, *C. gariepinus*

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2. INTRODUCTION

Aquaculture have contributed to the protein availability for human consumption despite age, class or sex [1]. Fish and fish products emanating from aquaculture practice surpasses that of the

marine capture fisheries with over 10,000,000 times [2]. Essential vitamins, fatty acids, minerals, lipids etc are contained in aquacultural products, and if consumed appropriately prevents the occurrence of certain diseases such as cancers, eye defects, cardiovascular disorders etc [3]. Pollution of our environment have contributed to decline in the fish availability [4], and aquaculturist and fish farmers must adopt a practice of not only improving the growth rate of fish, but also its survival. [5] observed that the type of feed administered in aquaculture dose not only determines the growth rate of fish but also the survival rate.

Herbs and herbal products have contributed to the growth and immunostimulant enhancement in aquaculture [6]. Some of the herbal products that have contributed to aquaculture practice includes: Avacado pear leaves aqueous extracts [7] Moringa leaves [8] and *Carica papaya* root extracts [9].

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Clarias gariepinus is an economically important ~~fresh water~~freshwater fish with outstanding qualities such as fast growth, diseases resistance, good flesh qualities among others [10].

Avocado pear (*P. americana)* leaves plants have been reported to be medicinal [11]. The introduction of avocado pear leaves in aquaculture practice has led to improvement in growth [12] and immunostimulants enhancement [13].

The purpose of this research work is intended to assess the medicinal phytochemical in the ethanolic extracts of the *P. americana* powdered leaves and the importance of powdered of the powdered leaves in the health of *Clarias gariepinus.*

3. MATERIALS AND METHODS

3.1 Experimental fish

The experimental fish was procured in Idi-onyana farms along Abua – Ahoada road in Abua/Odual Local Government Area of Rivers State.

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3.2 Experimental diets

Persea americana leaves were harvest in Port Harcourt, Rivers State. They were air dried to constant weight, grounded to it's powdered form, sieved and stored. The sieved powdered leaves were added to 38.35cp formulated diet (Do) at 3% (D1), 6%(D2), 9% (D3) and 12% (D4) respectively following the method of [14].

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3.3 Phytochemistry of Powdered P. americana leaf ethanol extracts

Quantitative analysis of powdered *P. americana* leaves was carried out in the Department of Chemistry, Faculty of Science, Rivers State University, in accordance with ISO 179245 using UV 250 visible.

3.4 Experimental procedure

~~One hundred and fifty (~~150~~)~~ catfish of 117.8± 1.11g and 25.88± 0.14cm weight and length respectively were stocked in the experimental tanks at 10 fish per tanks in triplicates, and feeding commenced 24 hours after stocking. The fish were fed the experimental diets according to the designated tanks (Do-D4) at 5% body weight per day, 2 times daily. After eight ~~(8)~~ weeks of feeding, the fish were injected three consecutive times (at day 1, 7 and 14) intraperitoneally with 1.5ml of 1.9 x 10^5cfu/ml of over night grown *K. pneumonia.* After 21 days from the first infection, blood samples were collected for haematological analysis, and the organs such as blood, liver, kidney and spleen were collected for microbial (*K. pneumonia*) presence analysis.

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3.5 Analysis of Plasma Haematological Parameters

Blood samples were collected from the experimental fish after day 21 days post infection via kidney puncture using an injection niddle and 5ml syringe. Samples were taking to the laboratory and analysed for packed cell volume (PCV), Haemoglobin (Hb), Red blood cells count (RBC), Thrombocytes (TCT) and White blood cell (WBC) using auto haematological analyzer model MY-BOO2B, while white blood differential and red blood indices were analysed using the methods of [15].

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3.6 Microbial Load (Anti-bacterial)

Approximately one gram of each organ was weighed independently and macerated in a universal container 10ml of normal saline was added to the macerated samples. 10ml of each diluted organs were cultured on TSA and inoculated at 37^{o}C for 24 hours. The number of bacteria were determined by counting the colonies grown on the agar plate.

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3.7 Data Analysis:

The collected data were analysed using SPSS statistics software 17.0 for windows. A one way analysis of variance (ANOVA) was employed to reveal significant difference between control and treated groups. Turkey's multiple comparism test was applied to separate treatments with significant difference [16].

4. RESULTS

Table 1 shows the phytochemical analysis of the powdered *P. Americana* leaves, using ethanol as solvent, it reveals the presence of Phenol, Carotenoids, Alkaloid, Flavonoid, Saponin, Steriod,

Coumarine and Proanthocyaniden at varying quantities. The result of the haematological parameters is in Table 2, the PCV, Hb, RBC and Neut were lower in the control group compared to the treated group. Basophil was zero across the experimental groups, the TCT fluctuated among the groups, but higher in D1-D3, but the WBC, Lymp, Eos, Mono, MCH, MCHC and MCV were higher in the control group compare to the treated groups. The result for the microbial load (Tables 3) reveals, that *K. pneumonia* presence was higher in the organs of the control group compared to the treated groups, while Plates 1 – 3 shows *P. americana* leaves at the fresh dry and powdered leaves respectively.

Table 1: Quantitative screening for Phyto-chemical Components of ethanol extract of Dry Avocado Pear Powdered Leaves (Mean ±SD)

COMPONENT	Yield (mg/dl)

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Phenol	43.1 ± 0.64
Carotenoids	0.851 ± 0.20
Alkaloid	0.50 ± 0.31
Flavonoid	3.60 ± 0.61
Saponin	1.30 ± 0.10
Tannin	0.00
Quinon	0.00
Steroid	1.31 ± 0.13
Triterpenoid	0.00
Coumarin	0.24 ± 0.12
Cyanogenic glycosides	0.00
Proanthocyanidin	0.93 ± 0.50
Phytocobillins	0.00
Pycocyanin	0.00

Data are mean ± Standard deviation of triplicate determination

Table 2: Haematological Parameters in Plasma of *C. gariepinus* fed Avocado Pear leaf Supplemented diets challenged with *Klebsiella pneumoniae* (Mean ±SD)

Parameters	Diets				
	D0	**D1**	**D2**	**D3**	**D4**
Packed Cell Volume (%) (PCV)	33.00±2.00 [b]	40.00±1.00[a]	43.00±2.00 [a]	44.00±1.00 [a]	44.00±1.00 [a]
Haemoglobin (g/dl) (Hb)	12.00±1.00 [a]	13.50±0.20[a]	14.40±0.10 [a]	14.53±0.21 [b]	13.50±0.20 [a]
Red Blood Cell (cells x 10^{12}) (RBC)	5.80±0.10[a]	4.60±0.10 [b]	6.47±0.15 [a]	6.47±0.15 [a]	6.00±0.10 [c]
White Blood Cell (cells x 10^{9}/l) (WBC)	16.40±0.10 [a]	10.00±0.20[b]	7.60±0.20 [c]	7.53±0.06 [c]	7.13±0.38 [c]
Neutrophils (%) (Neut)	27.00±1.00[c]	38.00±1.00 [b]	42.33±0.58 [a]	46.00±1.00 [a]	45.00±1.00 [a]
Lymphocytes (%) (Lymph)	60.00±1.00[a]	52.33±2.52 [b]	48.67±1.15 [c]	50.00±1.00 [b]	48.00±1.00 [c]
Eosinophils (%) (Eos)	4.00±1.00[a]	4.00±1.00 [a]	2.67±0.58 [b]	2.87±1.00 [b]	3.00±1.00 [b]
Monocytes (%) (Mono)	10.00±1.00[a]	6.67±0.58 [b]	7.33±0.58 [b]	5.00±1.00 [b]	5.33±0.58 [b]
Thrombocytes (%) (TCT)	215.00±1.00 [b]	217.00±2.00[b]	238.00±1.00 [a]	236.00±1.00 [a]	178.00±1.00 [c]
Basophils (%) (Base)	0.00±0.00[a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
Mean Corpuscular Haemoglobin (pg) (MCH)	36.52±4.72 [a]	33.77±1.19[a]	33.04±0.32 [a]	33.04±0.32 [a]	33.77±1.19 [a]
Mean Corpusc. Haemoglobin Conc. (g/dl) (MCHC)	26.08±1.97 [a]	23.28±0.66[a]	22.27±0.38 [a]	22.48±0.22 [a]	22.50±0.04 [a]
Mean Corpuscular Volume (FL) (MCV)	71.73 ±3.80[a]	68.96±0.54[b]	66.53±3.85 [b]	68.04±0.31 [b]	66.69±2.40 [b]

Means within the same roll with different superscript are significantly different ($p<0.05$)

Table 3: Microbial Load (cfu/ml) in Different Organs of *Clarias gariepinus* fed Avocado pear leaf Supplemented diets and infected with *K;ebsiella pneumoniae* (Mean ±SD)

Organs	Diets				
	D0	**D1**	**D2**	**D3**	**D4**
Kidney	56.33±7.57[a]	52.67±2.08[a]	43.67±1.52[b]	37.00±4.58[c]	11.33±3.51[e]
Spleen	42.00±2.65[a]	34.67±3.51[b]	21.33±3.01[c]	15.00±2.65[d]	4.00±1.00[e]
Liver	53.00±2.65[a]	35.33±2.52[b]	32.33±2.08[b]	28.33±3.06[c]	27.00±4.59[c]
Blood	90.00±4.36[a]	33.33±3.22[b]	33.33±2.08[b]	31.00±6.00[b]	19.67±5.03[c]

Means within the same roll with different superscript are significantly different ($p<0.05$)

Plate 1: Freshly harvested *P. Americana* leaves

Plate 2: *P. Americana* leaves air-dried to constant weight

Plate 3: *P. Americana* powdered leaves (grinded and filter)

5. Discussion

5.1 Phytochemicals of Avocado Pear Leaf

The ethanol extracts of the *P. americana* powdered leaves showed the presence of the following phytochemicals in varying quantities: phenol, saponin, carotenoids, alkaloids, flavonoid, coumarin, steride and proanthocyanidin. This result is in agreement with the findings of [17] and [18] who reported similar phytochemicals in the ethanolic extract of powdered *P. ameriana* leaves, but slightly different from the result of [19] who reported the absence of coumarin and alkaloids in the ethanolic extract of the dry Avocado leaves powder, probably due to experimental procedure.

Phytochemicals have been reported to have inhibitory and eliminating effects on series of micro-organisms that are life threatening to both humans, terrestrial and aquatic animals [20; 11]. The phytochemicals in powered Avocado leaves extracts are strong antioxidants that can prevent oxidative stress [21; 18] and modulate the metabolism of cholesterol in the body [22; 23], although saponins interupt the sucking up of minerals and vitamins in the body [24], the extracts of Avocado pear leaves have been observed to increase plasma enzymes and total cholesterol [25]. There may be more valuable and health benefiting phytochemicals in the avocado pear powdered leaves that were not identified by this quantitative screening, since studies have shown that the presence of phytochemicals in any extraction process depends also on the type of solvent used [18] and that ethanol is less effective in screening phytochemicals [26]. Methanol and water are most efficient solvents for the extraction of antioxidants [27]. The benefit of applying the powdered leaves in this research work is that all the phytochemicals in the leaves were completely utilized.

5.2 Effect of *P. americana* powered leaves on Haemotology of *K. pneumonia* C. *gariepinus*

The presence of bacterial infection alters the haematological paramaters of fish, and reveals, the health status of fish [12; 28]. After infecting *C. gariepinus* with *K. pneumonia* in this research work, the result indicated that the values of the PCV, Hb and RBC increased in the fish fed D1-D4 compared to the fish fed Do (control). This result is similar to the result obtained by [29] when *C. gariepinus* was experimentally challenged with *Escherichra coli* and *Vibrio fischeri*. The reduction in the PCV, Hb and RBC in the plasma of fish fed Do is an indication that the production and distribution of blood cells is negatively altered, and this could lead to low oxygen circulation in the fish [30]. The results agrees with the report of [31] who reported similar result when tilapia was naturally infected with *F. Columnaris*, and [32] who reported similar result when *channa punctatus* was challenged with *A. hydrophila*. The result of these parameters in the fish D1-D4 (Supplemented diets) after the infection shows that there were improved antibody production which promotes better oxygen circulation and blood cells formation [33; 34].

The WBC, lymp, Neut and Eos increased in the plasma of the fish fed Do compared to the fish fed D1-D4, this is an indication that the fish fed Do is under pathogen induced stress [35; 36], since these parameters are responsible for the protection of the fish against pollution, intoxicants, pathogen and other infections [37; 38]. The lower values of the WBC, lymp, Neut, Eos could be as a result of the presence of phytochemicals such as alkaloids, flavonoids, phenol etc. that are present in the *P. americana* powdered leaves diets (D1-D4), which have been reported to be bactericidal [11]. It could also be that the supplemented diets (D1-D4) reduced the pathogenicity of the *K. pneumonia* by boosting the immune systems of the experimental fish [39]. The fish fed

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D1-D3 had higher thrombocytes (TCT) values compared to fish fed Do after the infection with *K. pneumonia*. This result is in agreement with the result obtained by [28] when *C. gariepinus* fed dietary mango back was infected by *Pseudomonas geruginosa*. The improved TCT production in the fish fed D1-D4 could be as result of the phytochemicals in the supplemented diets (D1-D4) [40], the resultant effect could be the protection of fish against skin ulceration by bacterial infection [28; 41], since increase in thrombocytes prevents injuries and leads to quick recovery from external wounds [42].

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Basiphil was completely absent in all the experimental fish, this result is similar to the results reported by [31].

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5.3 Antibacterial activities of *P. americana* powered leaves

There were reduction in microbial load of the experimental fish as the percentage of *P. aeruginosa* powdered leaves increases in diets (Do-D4). The microbial loads were higher in fish fed Do compared to fish fed D1-D4. This result is in agreement with the one reported by [43] who reported reduction in the microbial load of *vibrio harveyi* infection when diets supplemented with herbs were administered to juvenile greasy groupers; and [44] who reported decrease in bacterial load in treated groups compared to the control when *C. gariepinus* fed various levels of onion bulb and walnut supplemented diets were exposed to *P. aeruginosa*. The reduction in the bacterial load in the fish fed D1-D4 could be as a result of the phytochemicals such as alkaloids, flavonoids, tannins, saponins present in the *P. americana* powdered leaves which have been reported to be immune-stimulant and anti-pathogenic [45; 11]. These phytochemical have also been reported to be bacteriostatic and bactericidal [46; 47].

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Conclusion

Persea americana powered leaves contains medicinal phytochemical that has prophylactic effect on the haematological parameters of C. *gariepinus* in the presence of *K. pneumonia*. The result of this work shows that *P. americana* powdered leaves is a strong antibacterial that can be used as a prophalytic to fresh water pathogens such as *K. pneumonia*.

Comment [N25]: Include some recommendations for future study

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