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Influence of *Phyllanthus muellerianus* aqueous leaves extract on haematological picture of African catfish (*Clarias gariepinus*)

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Abstract
Apart from the dwindling feral fish stock, human population is increasing fast. This requires more effort to produce fish at levels that will close the gap between demand and supply. The growth of a farmed fish is influenced by its health. One important parameter for health determination is the blood. Investigation on the influence of *Phyllanthus muellerianus* leaves extract on haematology of *Clarias gariepinus* was carried out. In this experiment, the fish of weight 77.33 $\pm$1.81 g and total length 21.72 $\pm$0.42 cm were exposed to different concentrations (0.0 mg/L, 8.0 mg/L, 16.0 mg/L, 36.0 mg/L, 65 mg/L, 130.0 mg/L and 260.0 mg/L) of the leaves extract. The extract was prepared by pounding the leaves and filtered. The filtrate was boiled to 100 ^{0}C for ten minutes. Ten fish each was stocked in 30 L of water containing different concentrations with three replicates. The culture lasted for 14 days with feeding at 6% body weight after which the blood was extracted with heparinized syringes and taken EDTA bottles to the laboratory. Blood was analyzed using computerized, automated hematological analyzer (Sysmex kx-21N™. The result showed that biological and behaviours of the fish were not different from that of the control. Among the physicochemical parameters, dissolved oxygen concentration conductivity and pH were lower in the treated groups. There was no change in temperature. The neutrophils and Lymphocyte did not differ due to treatment, the Monocytes, Basophils and eosinophils show significant difference due to exposure to *Phyllanthus* leaves extract ($\alpha = 0.05$). It is safe to conclude that *Phyllanthus muellerianus* leaves extract can be incorporated into the feeds of *Clarias gariepinus* for healthy growth.

Keywords: *Phyllanthus muellerianus, leaf* extract, haemo-immune status, *sub adult, Clarias gariepinus*, water quality

1. Introduction
Blood is an important tissue of fish. Its perfect performance as an organ is responsible for food distribution, disease fighting and cleansing by way of removing wastes from all parts of the body makes it important. Inability of blood to carry out these functions efficiently causes debility and reduction in growth of fish leading to reduced fish production. Many substances including phytochemicals could stimulate haemopoiesis. A plant *Phyllanthus muellerianus* has been found to improve blood and other health parameters in mammals especially man. The plants of the P*hyllanthus* genus are widely distributed in most tropical and subtropical countries including Nigeria, Cameroun, Ghana, Sierra Leone, Burkina Faso, Tanzania, Central Africa Republic, Congo, Cote d'Ivoire, etc. Because of its activities in blood of mammals [1], its influence on blood of fish could be investigated. If the result is favourable, there will be enhanced fish production due to faster growth and higher food conversion ratio (FCR). Improvement in fish production will reduce the gap of 900,000,000 metric tons (between demand and supply) of fish in Nigeria. We know that Nigeria is under pressure to meet 700,000 to 900,000 MT deficit of fish production requirement [2, 3]. According to these sources, Nigeria's current fish consumption is 7.5 kilogram fish per person against global value of 18.7 kilogram per person per year. Nigeria produces about 600,000 million tons of fish annually from Aquaculture and capture fisheries, but the country's shortfall is 900,000 million tons. The gap is currently reduced through importation at a value of 1,300 million US dollars per year. The need to boost fish production for ever increasing population all over the world cannot be over emphasized. This is achievable by increasing the growth rate of fish in culture environments in order to increase the turnover rate. Fish contributes 22% of animal protein supply in sub-Sahara Africa and 40% of animal protein consumption in Nigeria [4]. Several steps have been carried out to narrow the gap between production and demand.

Some of such steps include improvement in feeding of fish as well as increasing participation in fish farming. Feeds have been observed to consume a high proportion of the production cost in fish culture. Apart from good quality feeds, health status is of equal importance, and do enhance the utility of food. As with all animal production, disease is considerable constraint in production, development and expansion in the aquaculture industry. A fish that is not healthy does not feed well nor will it digest and absorb enough nutrients from the gut for fast growth. Several investigations have been carried out on the influence of xenobiotics on blood and general health of fish [5-9]. Literature on the use of commonly available herbal extracts to improve the health status of fish is sparse. Certain plants extracts used in human traditional medicine could be used to improve the health status of fish.

Though *Tilapia* is higher in the scale of most cultured fish in Nigeria, *Clarias* has higher advantage because it has good eating quality, command high price, rarely dies due to transportation stress, grow fast in captivity due to its acceptance of artificial feeds and can endure hash environmental conditions. It is comparatively low in natural food chain, due to its omnivorous feeding habits. It could be easily poly cultured with tilapia where it could keep tilapia population in check. Since the fish readily accepts artificial feeds, it becomes easy to modify the feeds to provide it with enhanced health and optimal growth as index of fish meat production. Such a species is promising in reducing lag between demand and supply of fish.

The plant *Phyllanthus muellerianus* is been used by traditional herbalists as blood builder and antibiotics. The genus *Phyllanthus* has a large number of medically important species for man. Some species are said to have potential effects against hepatitis B and antiviral activity against human immunodeficiency virus [10]. *Phyllanthus muellerianus* extracts are antimicrobial [11, 12].They are being used in the treatment of various liver disorders in India. In Thailand, *Phyllanthus amarus* (Schum. & Thonn) has also been widely used as an antipyretic, a diuretic, to treat liver diseases and viral infections [13].Their medicinal importance is due to the presence of the phytochemical compositions. They have been observed to contain the following active ingredients: alkaloids, coumarins, flavoids, phenols, steroids, saponins, triterpenoids, glucosides and dihydrochalcones [14].

Since this plant has been evaluated to boost the immunity through blood building as well as keeping the animal free of parasites in mammals, it is pertinent to investigate its effects on the blood of cat fish which is generally cultured and highly acceptable for consumption in Nigeria. It is known that the health of any vertebrate could be seen from its blood picture [15].

2. Material and methods

2.1 Specimens collection and acclimation

Sub adults of *Clarias gariepinus* of average weight 77.33 $\pm$1.81 g and total length 21.72 $\pm$0.42 cm were obtained from the Faculty of Agriculture and Forestry fish farm, Obubra campus of CRUTECH, Cross River State. These were transported in plastic buckets between 7.00 and 9.00 of the day to the laboratory for acclimation for a period of two weeks

The *Clarias gariepinus* were fed at 6% of their body weight. The feeds of pellet size 1.8 to 2mm were obtained from COPPENS, sold in Calabar, Nigeria. The daily ration was divided into two (3% body weight) and fed at 10 am and 4 pm [15, 17].

2.2 Culture system

Plastic aquaria of 52 cm length, 38 cm width and 30 cm height were filled with stream water up to 15.2 cm level giving a volume of 30 liters of water per tank. These were subjected to five different concentrations of the extract as described by Beitlich (1995) and (1997) in manual of aquatic Science research [18]. One treatment level had zero concentration of extract and served as control. Ten fish were selected randomly and stocked in each aquarium [15, 19-21].A semi static bioassay method was used in the various experiments. These experiments were replicated three times for each concentration [21]. The procedures for range finding test was repeated. But the highest concentration in definitive test was derived from the concentration, which did not kill any fish in the range finding tests.

2.3 Administration of leaves extract

Leaves were pounded and weighed. This was dissolved in water and then filtered. The filtrate was boiled for about ten minutes as it is the practice for its traditional herbal decoction in human medicine. Concentration of extract was given by weight of whole leave pounded minus weight of shaft/volume of water. These were measured in mg/L and added to30 liters in the culture aquaria. The concentrations used were 0.0 mg/L, 16 mg/L, 33 mg/L, 65mg/L, 130 mg/L and 260 mg/L. Ten fish were stocked per tank and each treatment had three replicates.

2.4 Water quality parameters

Temperature was measured using mercury in glass thermometer and electronically by Mettle Toledo 320 to the nearest degree Celsus. Oxygen and pH were also measured using electronic method. The pH meter model Mettle Toledo 320 was used for pH determination and oxygen.

The mean lethal concentration (LC_{50}) for 24 hours, 48 hours, 72 hours and 96 hours were computed using probit and logit [21-25]. The haematological parameters were analysed using analysis of variance (ANOVA) at 0.05 alpha level by SPSS, version 13.0. The *post hog* analysis was carried out using Duncan multiple range test.

3. Results

The biological observations were as expressed in Table 1. This shows that there was no change in the behaviours of the fish compared to the behaviours in the natural environment (control). That means that the inclusion of the plant leaves extract did not affect the biological behaviours of the fish.

Table 1: showing biological behavior of catfish, Clarias gariepinus juvenile (Definitive Test)

Behavior/ Exposure Time	**48hrs**						**72hrs**						**96hrs**					
Conc. (mg/L)	0.0	16.00	32.00	65.00	150.00	300.00	0.0	16.00	32.00	65.00	150.00	300.00	0.0	16.00	32.00	65.00	150.00	300.00
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molting	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Air gulping	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erraticswimming	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Key = present
= not present

Dissolved oxygen was significantly lower in treatments five and six with the highest concentrations of the extract. The extract may have possibly used up some dissolved oxygen. There were significant differences in hydrogen ion concentration and conductivity of the water, which was lower in the treatment groups. It is possible that these two parameters are influenced by the extract. See Figures 1, 2 and 3 respectively.

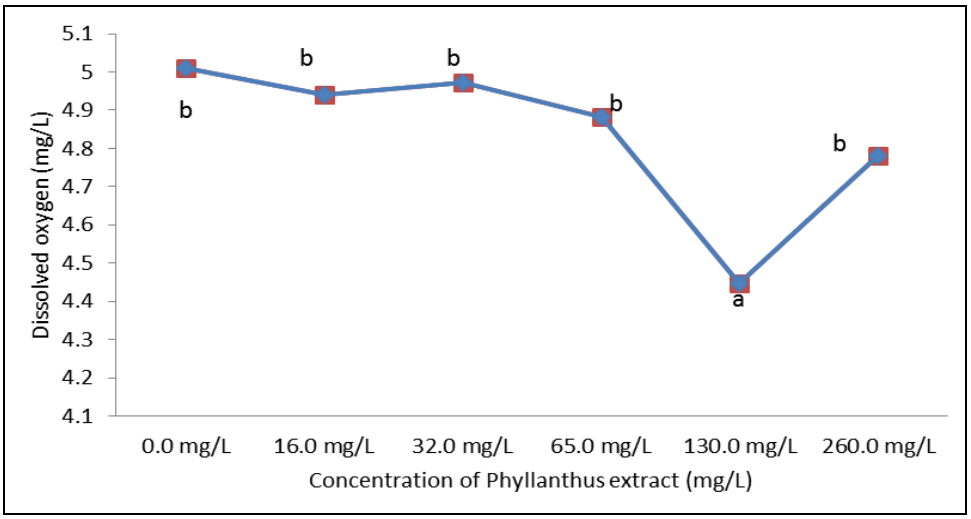

Fig 1: Dissolved oxygen was not significantly different in treatment groups compared to control except in treatment 5 with extract concentration of 130 mg/L.

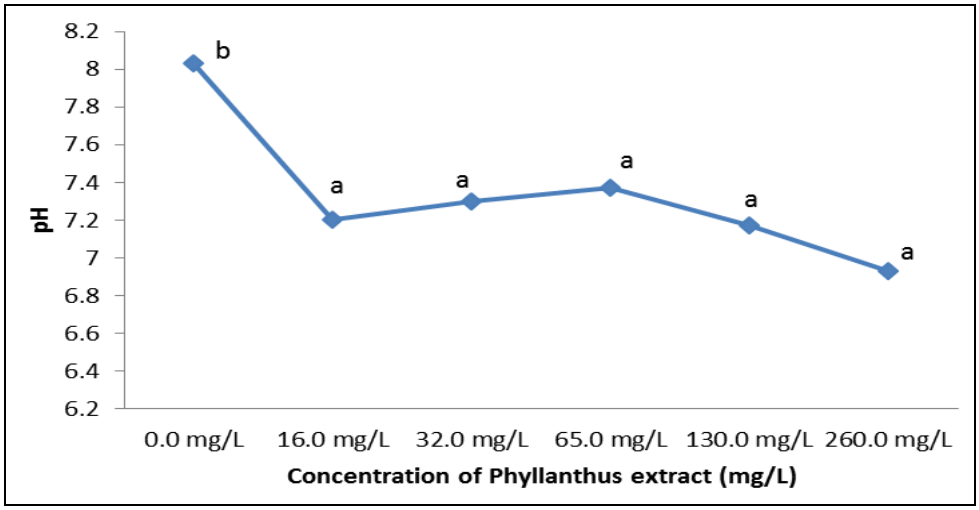

Fig 2: shows hydrogen ion concentration as influenced by extract concentration, which was lower in treatment groups compared to control. All means with the same letter are statistically the same while the control group alone has a different letter showing that it was different from others ($\alpha = 0.05$).

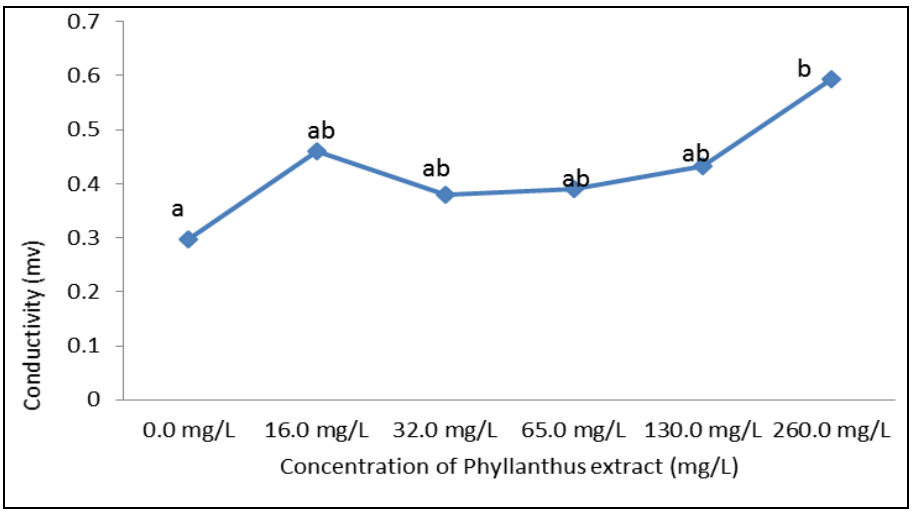

Fig 3: shows that there was increase in conductivity of water when treated with *Phyllanthus muellerianus* extract. This was observed to be higher with increase extract concentration. Means with same letters are statistically the same while those with different letters are different ($\alpha = 0.05$).

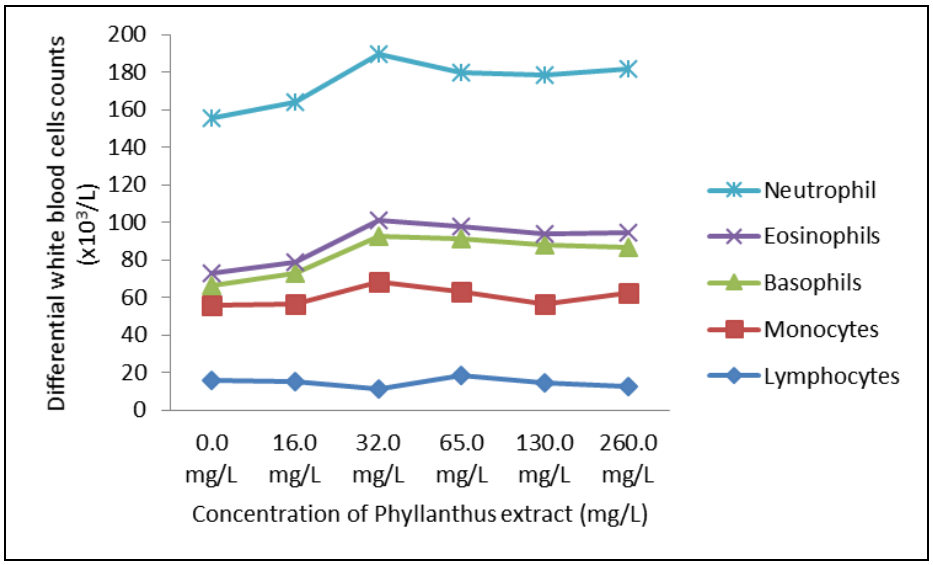

Fig 4: shows the concentrations of Neutrophils, Eosinophils, Basophils, Monocytes as well as the Lymphocytes (x10^3/L) counts in *Clarias gariepinus* exposed to different concentrations of Phyllanthus muelarianus extract. While the neutrophils and lymphocyte did not differ due to treatment, the monocytes, basophils and eosinophils show significant difference due to exposure to *Phyllanthus* leaves extract (α = 0.05).

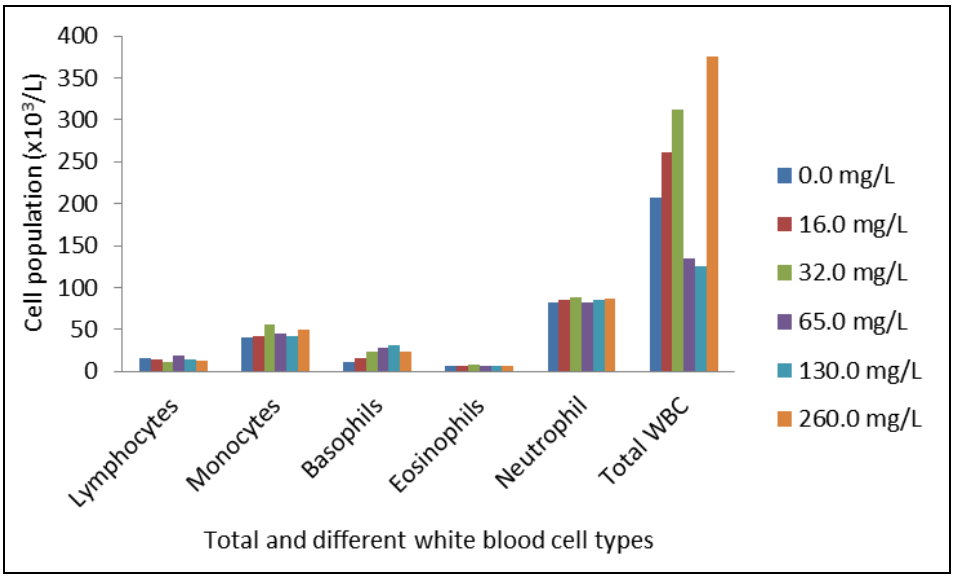

Fig 5: shows different white blood cells counts (x10^3/L) in comparison with the total. Total white blood cells count (x10^3/L) were significantly different among treatments (α = 0.05)

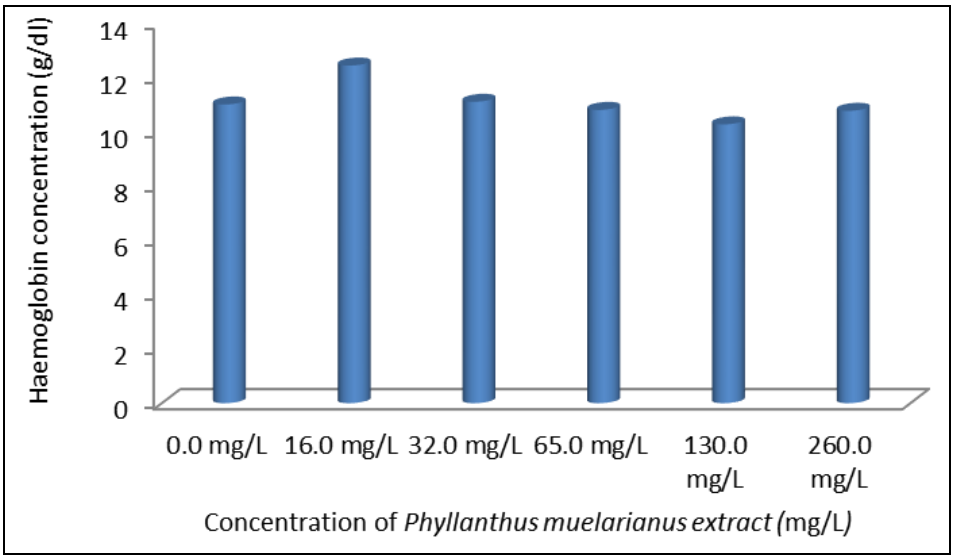

Fig 6: Haemoglobin concentration (g/dl) of *Clarias gariepinus* sub adults did not changed significantly when exposed to *Phyllanthus muellerianus* leaves extract (α = 0.05)

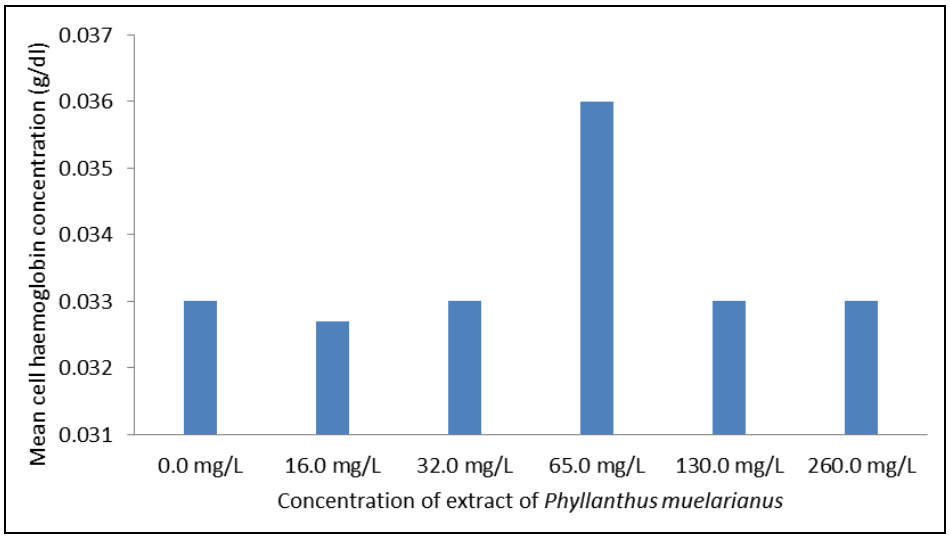

Fig 7: Mean cell haemoglobin concentration (g/dl) in *Clarias gariepinus* sub adults exposed to leaves extract of *Phyllanthus*. There was no significant difference between treatment and control groups (α = 0.05).

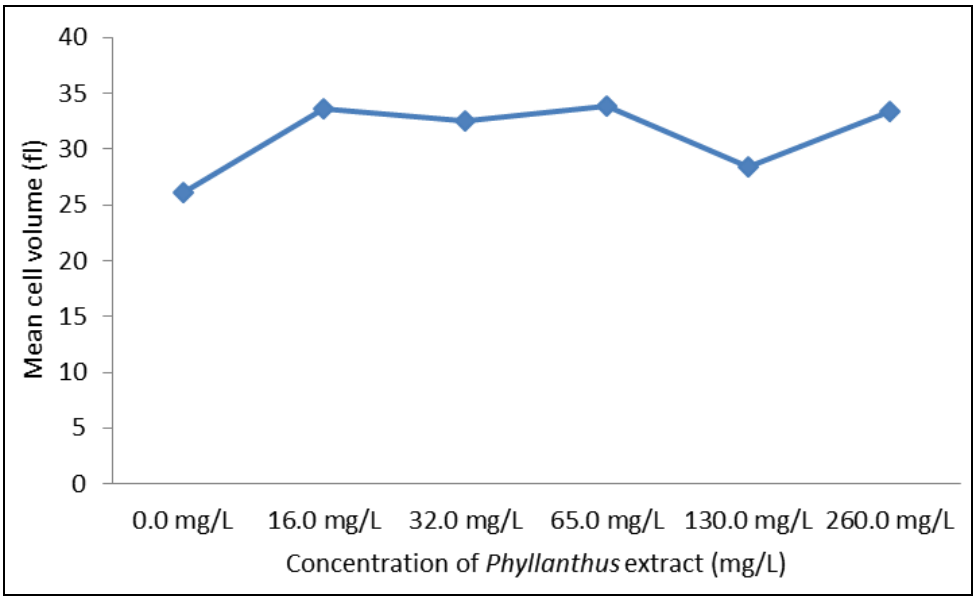

Fig 8: showing mean cell volume in sub adult *Clarias* exposed to leaves extract of *Phyllanthus* plant. There was no significant difference among means (α = 0.05)

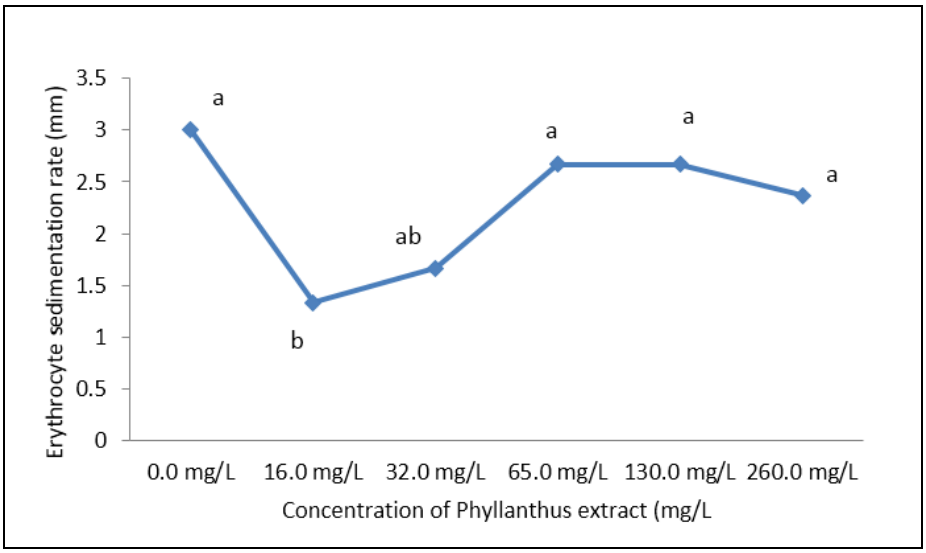

Fig 9: showing rate of red blood cell sedimentation (mm) in *Clarias gariepinus* exposed to *Phyllanthus muellerianus* leaves extract. There was a significant difference between the treatments (α = 0.05). Means which carry the same letters were statistically the same while those with different letters are statistically different. Comparing this graph with Figure 5 revealed that the mean cell volume and erythrocyte sedimentation rate are moving in inverse directions, though the mean cell volume was not significant among the treatments

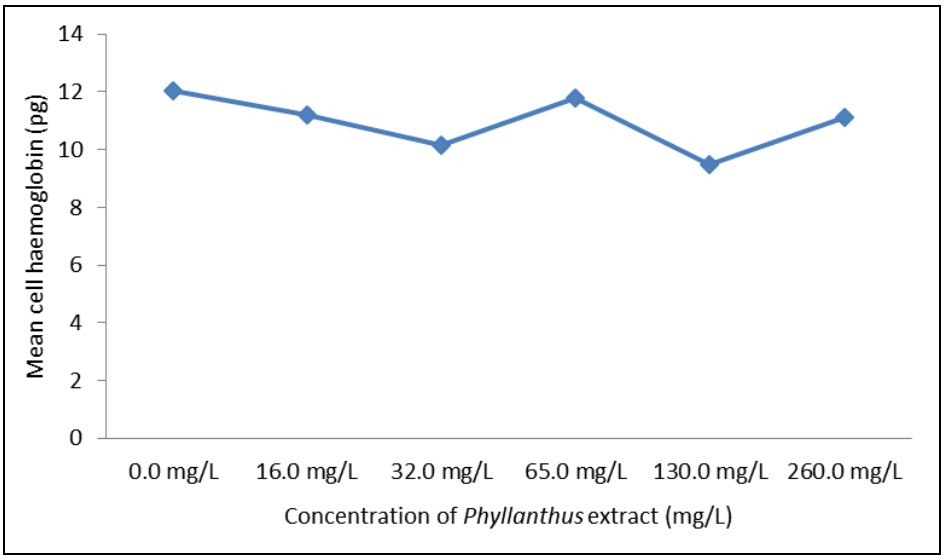

Fig 10: the mean cell haemoglobin of sub adult *Clarias gariepinus* did not show any statistical difference when the fish was exposed to different concentration of *Phyllanthus muellerianus leaves* extract

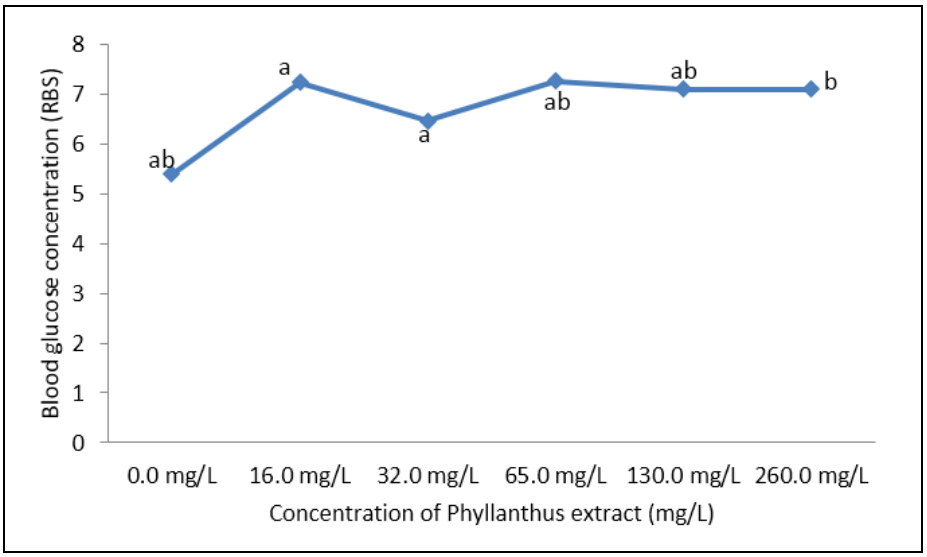

Fig 11: Blood sugar level (Glucose concentration) in *Clarias gariepinus* exposed to leaves extract of *Phyllanthus muellerianus.* Concentration in all the treatment groups were significantly higher than the control group ($\alpha = 0.05$).

4. Discussion

There was lower dissolved oxygen in one treatment and may be attributed to the treatment since values below and above this treatment indicated the same decreasingtrend with concentration of extract, though were not significantly different from the control group.

In order to increase productivity, profitability and sustainability, there is need to boost the haemo immune status of this important fish using available substances that could serves this purpose. Decoction from the leaves of *Phyllanthus muellerianus* is likely to promote these values. Xenobiotics such as tannins, flavonoids, saponins, alkaloids and anthraquinones are present in the phytochemical screening of the leaves and stem bark of P*hyllanthus muellerianus* [26, 27], these can be used to improve the immune status of African catfish. Phytochemicals like ellagitannins geraniin, corilagin, furosin the flavonoids, common plant pigment compounds present in the plant act as antioxidants that enhance the effects of vitamin C. These are capable of strengthening connective tissue around capillaries. All the parts of this plant (root, shoot, leaves, branches and stem bark) are widely used to treat intestinal problems such as constipation, toothache, eye pain, sore throat, jaundice, fever, paralysis, pneumonia, dysentery, diarrhea and stomach problems. These have made it a plant of interest by researchers to evaluate its potential for fighting diseases by boosting haemo immune status of organisms such as man. This work has shown that it can be applied to lower organisms such as fish. Unpleasant environment or predisposing factors leading to stresses such as temperature, overcrowding, organic pollution, and hypoxia leads to decrease in the haemo immune status of fish. Research done on these plants has found that the derived compounds of *Phyllanthus muellerianus* acts as a better immune-stimulant, antibacterial, antiviral, and anti-stress in fish and shellfish aquaculture [1, 19]. Gallate is an antioxidant used in fatty food preservation. Literature reveals that propyl, octyl and dodecylgallate. caffeic acid, chlorogenic acid. 3.5-dicaffeoylquinic acid and caffeoylmalic acid (phaselic acid) have been identified in *P. muellerianus*, with Geraniin dominating in aqueous extract [1]

According to Ben-Bala [26], both the aqueous and methanol extracts of the leaves and stem bark showed high antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. A chloroform extract showed high antifungal

activity against *Candida albicans* and antibacterial activity against *Escherichia coli*. This antimicrobial/antifungal effect is capable of reducing stress due to microbial infections, thus, causing the fish's metabolism to function efficiently.

So, its healing activities could be derived from its antimicrobial effects [28]. This plant's haematological enhancing power could be due to its ability to eliminate microbial infections that often parasitizes blood cells and the related tissues. On relieving the fish of its parasite burden, digestion, assimilation, as well as metabolism can go on undisturbed. Haemopoiesis is an integral part of this. Haemopoiesis in a parasite free body could result in the production of proportionate blood cells types capable of fighting infection and transport and distribution of gases, nutrients, hormones and wastes.

Blood cell responses are important indicators of changes in the internal and/or external environment of animals [27]. Their changes depend on fish species, age, the cycle of the sexual maturity and state of health [29-31]

Blood sugar was observed to be raised in treatment groups. Hattingh [32] observed blood sugar increases in *Labeo capensis* exposed to some environmental factors such as reduced oxygen concentration, increase in ammonium level, transportation and capture related stress. In this work, *Clarias gariepinus*, blood sugar was observed to be increasing with conductivity. Glucose concentration is not necessarily due to greater availability of glucose in feeds or food, but could be influenced by rate of absorption and insulin availability. *Phyllanthus* leaves extract may have enhanced glucose absorption from gut [33]. At normal concentration and increase availability, sugar is the fuel of the body to provide strength for the fish to carry out other life processes as respiration, growth, reproduction, tissue repair and even movement.

5. Acknowledgement We are grateful to Mr. IgriIgri of The Central Laboratory, Faculty of Agriculture, University of Calabar, Nigeria for haematological analysis.

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