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Effects of herbal mixtures (*Jedi, Gbewutu* and *Opa-eyin*) on the health status of juvenile african catfish (C*larias gariepinus*)

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ABSTRACT

The study was conducted to establish the effect of some herbal mixtures: *Jedi, Gbewutu and Opa-eyin* on the health status of juvenile *Clarias gariepinus* (49.20±1.59 g) using hematological parameters and growth indices. A total of 150 fish were divided into 3 groups (50 individuals per group) and each group was fed with varied levels of *Jedi, Opa-eyin* or *Gbewutu* (0- control, 5, 10, 15, and 20 ml/g feed) for 40 days. The fish in the treatment with *Jedi* and *Gbewutu* herb exhibited significantly higher food intake, hence mean weight gain, final weight gain, specific growth rate, and relative weight gain that the control. The white blood cells and the packed cell volume in the control fish fed with either herb was also higher than the treatments. The reverse was the case with hemoglobin and mean corpusculat hemoglobin in which the levels were lower in th control than the treatments. Results of the growth indices and feed intake suggests that the appetite of the fish, particulalrly those fed on *Jedi* diets might have reduced with progressive inclsuions. Furthermore, mobilization of the white blood cells and neutrophils in response to the inclsuions suggests that the immune system of the fish might have been sentitized by the herbs, suggesting toxicity. *Opa-eyin* didn't impair the growth of the fish on the basis of incremental inclusions. Results indicate that the herb might have improved the feed conversion rate of the fish. *Jedi* > *Gbewutu* herbs possess toxic tendencies and might be counterproductive in animals and humans. The study suggests that Opa-eyin has promising therapeutic potentials and might be an effective feed inclusion.

INTRODUCTION

The use of herbs is an old practice, dating back to ancient times (Fabricant and Farnsworth 2001; John, 2001; Mahajan *et al.*, 2013; Mohd-Setapar *et al.*, 2016; Brima 2017). The World Health Organization (WHO) estimates that up to 80% of the world's population relies on traditional medicine system for some aspects of primary health care (Gu *et al.*, 2013) and knowledge about this traditional medicinal practice is transmitted from generation to generations (Vermal *et al.*, 2011). The herbal drugs are used not only against diseases but also as growth promoters, stress resistance and prevention of infections (Madhuri *et al.*, 2012). Herbs are also used as antistress, appetizer, antimicrobials and immunostimulants (Citarasu *et al.*, 2010). Studies have shown that herbs are used in Nigeria as treatment for malaria, blood sugar control,

diarrhea, dysentery, dysmenorrhea, and cholera (Patrick, 2002; Somjetlerdcharoen, 2002; Oreagba *et al.*, 2011).

However, the scientific community, particularly the pharmaceutical scientists have not ascertained or approved the overall quality, safety and efficacy of many herbal products consumed by the Nigerian populace (Moreira *et al*., 2014).

The efficacy of some plants is inherent in some of the chemical substances they synthesize (Edeoga, 2005) and many herbs are consumed regardless of invalidation by the regulatory agencies (Elujoba, 2005). In most cases, medicinal value of the herbal products is merely based on beliefs and not supported by scientific data (Patrick, 2002). Hence, cardiotoxicity, nephrotoxicity, carcinogenicity and mortality may be associated with the use of herbal medicines (Ernst, 2003; Lans, 2006; Tovar and Petzel, 2009; Van Hai 2015).

Furthermore, lower price, greater accuracy and lower side effects compared to additives, inform aquaculturist's preference for the use of herbal products for promoting animal health and production (Citarasu 2010; Ghasemzadeh *et al.*, 2010; Tatina, 2010; Ghasemzadeh and Ghasemzadeh 2011; Govind, 2012; Syahidah *et al.*, 2015). Herbs have often served as an alternative to antibiotics in fish health management. Many studies have proven that herbal additives has the potential to enhance the growth of fishes and protected them from diseases (Johnson and Banerji 2007; Harikrishnan *et al.*, 2011; 2012).

It has been documented that some plant extracts exhibit antistress, growth promotion, appetite stimulation, immunostimulation, aphrodisiac and antipathogen properties in fish and shrimp aquaculture due to their varied active components such as alkaloids, terpenoids, tannins, saponins and flavonoids (Citarasu, 2010; Chakraborty and Hancz, 2011). The synergistic effect of herbs has been reported in many fishes, especially *Clarias gariepinus* (Turan and Akyurt, 2005).

Hematological indicators such as hematocrit, hemoglobin, erythrocyte and lymphocyte levels can be used to determine fish fitness (Haghighi and Rohani 2013). Fish immunity is often analyzed to determine the immunomodulatory power of plants and this can be studied via classic biochemical approaches such as lysozyme, phagocytic or respiratory burst activity or by the study of immune gene expressions such as Lys, TNF-alpha, IL-1, IL-10 genes (Harikrishnan *et al.*, 2011; Kumar *et al.*, 2013; Chakrabarti *et al.*, 2014). High hematocrit is used as an indicator of the excessive intake of exogenous erythropoietin (EPO), which stimulates the production of red blood cells.

Histopathological changes and oxidative stress have been increasingly studied as biomarkers for assessing aquatic contamination in environmental monitoring studies (Kelly and Janz, 2009; Ben Ameur *et al.*, 2012; Fricke *et al.*, 2012). The potential for the application of research findings to both human and environmental health issues makes fish species attractive and valuable alternative models in the carcinogenesis and toxicity research. African catfish (*Clarias gariepinus*) is known for its ability to perform aerial respiration which enables them to migrate through deoxygenated swamps and pools, as well as over land. This wide range environemental adaptability makes them a good experimental candidate.

Considering the ecofriendly, immunomodulating properties and the toxicity or inhibitory potentials of herbal supplements, this study thus investigates the hematological, biochemical and histopathological effects of herbal concoction (named *jedi*, *gbewutu* and *opa-eyin* in Nigeria) at varying inclusion levels on juvenile *Clarias gariepinus*, to test the safety level based on hematological and biochemical indices.

MATERIALS AND METHODS

The experiment was carried out at the feed processing unit of the Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka. The composition of the polyherbal mixtures used traditionally mainly in Sub-Sahara Africa for human health care called *Jedi, Opa-eyin,* and *Gbewutu* in Nigeria are presented in Table 1.

Table 1: Constituents of polyherbal mixtures studied

S/N	Name	Constituent	Family	Local Name	Folklore	Native Countries	Refernces
1	Jedi	*Khaya ivorensis*	Meliaceae	African mahogany "Oganwo"inYoruba	Antibacterial, Antifeedant,antifungal,and for dysentery.	Angola,Benin, Cameroon,Cote d'ivoire,Gabon, Ghana,Togo, Fiji,Indonesia and Malaysia.	Neuwinger, (2000) ; Vanucci *et al.* (1992).
		Senna fistula	Fabaceae	Indian laburnum Aidantoro in Yoruba	Antiviral, Antidiabetic, Antiulcerogenic, Antioxidant,Hepatoprotective ,diabetes and infertility.	India and Sri lanka	Senthil *et al.*, (2006).
		Axonopus compressus	Poaceae	carpet grass "ewe idi"in Yoruba	Malaria, fever, asthma, and postnatal breast treatment.	Nigeria	
		Lecniodiscus cupaniodes	Sapindaceae	Planch "Arika" in Yoruba	Inflammatory conditions, hepatomegaly,and bacterial infections	Uganda Sierra Leone ,Sudan and Angola.	Gill (1992); Iwu, (1993),Yemitan and Adeyemi, (2004).
		Hunteria umbellate	Apocynaceae	Spanish needle "abeere" in Yoruba	Fish poisoning and treatment of high blood pressure. used to treat diabetes, hypertension,and constipation.	Central African Republic, Zaire and Nigeria.	
		Gongronema latifolium	Asclepiadaceae	Bush buck "Madunmaro"in Yoruba.	used against cancers and tumors, tuberculosis, indurations of liver and spleen.	Nigeria, Sierra Leone and Ghana.	Edet *et al.* (2011).
		Acacia nilotica	Fabaceae	Gum Arabic "booni" in Yoruba.	for culinary purposes and medicinal purposes.	Asia, Australia, America and Africa.	Kalavani and Matthew (2010): Solomom-Wisdom and Shittu (2010).
		Monodora tenuifolia	Annonaceae	African nutmeg "Ariwo"in Yoruba.	used to cure hernia, wounds, haemorrhoids, ulcers, sores, leprosy. gastric pains, diarrhea, oedema,,paralysis, epilepsy and madness.	Nigeria, Zaire, Guinea, Ghana, Sierra Leone.	Burkil (2004).
		Lannea egregia	Anacardiaceae	"Ekudan"in Yoruba	used to treat diarrhea, rheumatism, jaundice, venereal diseases and snakebites	Guinea, Ivory coast, Dahomey and Nigeria.	
		Rauvolfia vomitoria	Apocynacea	Poison devil's pepper "Orira" in Yoruba		Senegal,Ugan,Tanzania, Congo and Angola	Neuwinger (1996)
2	Gbewutu	*Cochlospermum tinctorium*	Cochlospermaceaae	"Gbewutu" in Yoruba	Used against pains, fever, maleria and inflammatory condition	West Africa	Usman et al. (2008), Fernsworth (1998).
3	Opa-eyin	*Senna fistula*	Fabaceae	Indian laburnum "Aidantoro" in Yoruba	Antiviral, Antidiabetic, Antiulcerogenic, Antioxidant,Hepatoprotective ,diabetes and infertility.	India and Sri lanka	Senthil *et al.*, (2006).
		Chasmathera deperdens	Rutaceae	"Atoo" in Yoruba		Nigeria.	Lamidi et al (1995).
		Carpolobia lutea	Polygalac Eae	Cattle stick "Ikpafun" in Ibibio	Epilepsy,bone setters Sexual Dysfunction,madness and infertility	Nigeria, Ivory coast	Yakubu and Jimoh (2015).
		Lecaniodiscus cupanioides	Sapindaceae	"Akika" in Yoruba		Egypt, Greece,Sri Lanka and Nigeria.	Fasuyi 2006 and Mensah *et al.* (2008).
		Crossandra puberula	Acanthaceae		Cholera,fever,bowel,ulcer,leprosy,skin diseases and menstrual problem	Tanzania,Malawi ,Zimbabwe	Da Silva *et al.* (2004).
		Aristolochia indica	Aristolochiaceae	Creeper plant		Indian, Sri lanka	Murugan *et al.* (2006).

Collection of samples

Fresh samples of *Jedi*, *Opa-eyin* and *Gbewutu* were sourced at a local market in Mushin, Lagos state, Nigeria. The herbal concoctions were collected in clean sampling bottles and preserved in ice chest coolers, in which they were transported to the laboratory of the Department of Marine Science, University of Lagos, Nigeria, where they were refrigerated at 4 ^{o}C prior to commencement of the experiment.

Toxicity test

Juvenile *Clarias gariepinus* (49.20- 50.02 g) were held in transparent plastic tanks (52 cm x 33 cm x 21 cm). The fish were acclimatized for 14 days at water temperature of 23-25°C and pH 7.0-7.2 in 12-12 hr light-dark photoperiods. They were fed with commercial diet before the introduction of experimental diet.The water was changed 48-hourly (Finney, 1952; Aderolu and Akpabio, 2009).

Experimental design

A total of 150 juvenile *Clarias gariepinus* were recruited in the experiment. They were properly screened for diseases and fitness prior to purchased from Iceberg Agricultural Consult Limited, Governor Road, Ikotun, Lagos. The fish were divided into 3 groups (50 individuals per group) and each group was fed with *Jedi*, *Opa-eyin* or *Gbewutu* for a period of 40 days.

Feed formulation and feeding regime

All other feed ingredients were sourced from Solace Nigeria Limited, Oko-Oba Agege, Lagos, Nigeria. The feed ingredients obtained include fish meal, soybean meal (SBM), groundnut cake (GNC), maize, noodles, di-calcium phosphate (DCP), premix, and lysine, methionine, vitamin C and salt (NaCl).

Five diets were manually formulated for the different herbal concoction (Table 2). The *Jedi*, *Gbewutu* and *Opa-eyin* were separately added at graded levels to the feed to form Diet 1 (feed only- control), Diet 2 (5 ml/g feed), Diet 3 (10 ml/g feed), Diet 4 (15 ml/g feed), and Diet 5 (20 ml/g feed). These inclusions were thoroughly mixed with the formulated feed and pelletized using a pelletizing machine (2 mm). The moist pellets were sun dried for 8 h, packaged in air tight plastic containers, tagged and refrigerated until needed.

In each of the 3 groups, comprising 50 individuals each, 5 sub-groups comprising 10 individuals each were separately fed Diets 1- 5 in different tanks. In total, 15 tanks were designated to all the clusters in the entire feeding regimes under the 3 large groups (*Jedi*, *Gbewutu* and *Opa-eyin*). They were all gavaged thrice daily, in the morning (8:00 am), afternoon (1:00 pm) and evening (5:00 pm). The fish were monitored for mortality on a daily basis and dead fish were removed weighed and recorded. Water in the tank was changed every other day to maintain good water quality as described by Aderolu and Akpabio (2009).

Table 2: Feed formula ingredients and nutritional information

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet5
Fish meal(kg)	20.00	20.00	20.00	20.00	20.00
Soybean meal (kg)	24.50	24.50	24.50	24.50	24.50
Groundnut cake(kg)	18.00	18.00	18.00	18.00	18.00
Maize(kg)	23.00	23.00	23.00	23.00	23.00
Indomie waste (kg)	12.00	12.00	12.00	12.00	12.00
Dicalciumphosphate(kg)	0.50	0.50	0.50	0.50	0.50
Methionine(kg)	0.25	0.25	0.25	0.25	0.25
Lysine(kg)	0.50	0.50	0.50	0.50	0.50
Premix(kg)	0.50	0.50	0.50	0.50	0.50
Vitamin C(kg)	0.25	0.25	0.25	0.25	0.25
Salt(kg)	0.50	0.50	0.50	0.50	0.50
Extract (cl)	-	5	10	15	20
TOTAL(kg)	100	100	100	100	100
NUTRIENT VALUE					
Crude Protein(%)	35.61	35.61	35.61	35.61	35.61
Energy (Kcal/g)	2873.4	2873.4	2873.4	2873.4	2873.4

Growth and nutrient utilization parameters

The weight gained and nutrients supplied were computed every 10 d and later used to compute growth, feed utilization and economic parameters. For this study, growth was expressed as mean weight gain (MWG), relative weight gain (RWG) and specific growth rate (SGR) according to Morais *et al.* (2001):

1)Mean weight gain (MWG) (g) = Mean final weight (g) - Mean initial weight (g)

2)Relative weight gain (RWG) = $\frac{\text{Average weight gain (g)}}{\text{No of days (days)}}$

3) Specific growth rate (SGR)= $\frac{\text{Loge W2 (g)- Loge W1 (g)}}{\text{T2-T1 (day)}}$ x 100

Nutrient utilization indices were expressed as feed conversion ratio and protein efficiency ratio as follows:

4) Feed conversion ratio (FCR)

FCR=$\frac{\text{Feed eaten in dry mass (g)}}{\text{Weight gain (g)}}$

5) Protein efficiency ratio (PER)

PER = $\frac{\text{Mean weight gain (g)}}{\text{Protein intake (g)}}$

Protein Intake (PI) = Total feed intake x Protein content of feed

Hematological analysis

Effects of dietary treatments on the haematological profile of *Clarias gariepinus* at the end of the 40 days feeding trials were analyzed. The fish were sedated with 80% ethanol, after when they were euthanized. Blood samples were collected with the aid of 2 ml syringes from the caudal vasculature of the fish from each treatment group and emptied into Heparin bottles for hematological and biochemical analyses. The hematological samples were analyzed in the Department of Medical Laboratory Sciences, Lagos University Teaching Hospital (LUTH) for hematological parameters.

Hematocrit and erythrocyte count determination

The hematocrit (Hct) was used to determine the packed cell volume (PCV) in the blood. The analysis was carried out to determine the correlation between hemoglobin content and erythrocyte count and also to determine the relative

proportion of the red blood cell in the blood of the fish. In normal conditions there is a linear relationship between hematocrit and the concentration of hemoglobin (*c*tHb). An empirical study has shown that the relationship can be expressed thus:

Hct (%) = (0.0485 × *c*tHb (mmol/L) + 0.0083) × 100.

Hematocrit (PCV) was estimated from measurements of the mean cell volume (MCV) or the mean corpuscular hemoglobin concentration (MCHC):

Hct (%) = MCV × RBC × 0.1

Hct (%) = *c*tHb × 100 MCHC

Normally PCV is about 45%, that is, the packed cells account for 45 ml in 100 ml blood.

Hemoglobin content (Hb) determination

Hemoglobin content was tested using hemiglobicyanide, a spectrophotometric method (ICSH, 1967; 1996). Twenty five (25) µl of the blood sample was diluted in a solution containing 200mg potassium ferricyanide ($K_3Fe(CN)_6$), and 50 mg potassium cyanide (KCN), dihydrogen potassium phosphate (KH_2 PO_4), and 1 ml non-ionic detergent; all diluted with 1000 ml distilled water (Van Kampen and Zijlstra, 1961). The mixture was agitated and allowed to stand for 3 minutes. $K_3Fe(CN)_6$ oxidized the iron in heme to the ferric state to form methemoglobin, which was converted to to hemiglobicyanide (HiCN) by KCN (ICSH, 1967). The absorbance of HiCN was read at 540 nm against a reagent blank. The absorbance of HiCN standard was then measured in the same way.

Statistical analysis

All data procured over the duration of experiment were subjected to one-way analysis of variance (ANOVA). Comparisons among treatment means were carried out using Tukey Multiple Range Test (Tukey, 1951) at a significant level of $p < 0.05$. All computations were conducted using the statistical package PASW/SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Toxicity test

The toxicity (LC_{50}) tests of the herbal concoctions were performed in quadruplets. The *Jedi* tanks (pH- 2.7) recorded 100% mortality in tank with 300 ppm at 3 h, 25% mortality in tank with 150 ppm at 1 h and 75% mortality in tank with 75 ppm at 2 h (Table 3). No mortality was recoded in tank with 37.5 ppm. Gbewutu (pH-4.8) recorded 50% mortality in tank with 300 ppm at 3 h and no mortality was recorded in other tanks.

Opa-eyin (pH- 5.8) recorded 50% mortality in tank with 300 ppm at 5 h and 25% mortality in tank with 150 ppm, 75ppm and 37.5 ppm at 6, 7, and 8 h respectively.

Table 3: Percentage mortality of juvenile *Clarias gariepinus*

Herb type	Conc. (ppm)	Log 10	% Mortality	Probit
Jeddi	37.5	1.574031	75	5.67
	75	1.875061	0	0
	150	2.176091	25	4.33
	300	2.477121	100	3.75
Gbewutu	37.5	1.574031	0	0
	75	1.875061	0	0
	150	2.176091	0	0
	300	2.477121	50	5
Opa-eyin	37.5	1.574031	75	5.67
	75	1.875061	0	0
	150	2.176091	25	4.33
	300	2.477121	100	3.72

No significant difference occurred in the initial weight gain (IWG) among the different treatments of *Jedi* (Table 4). However, there was a constant significant decrease in the feed intake (FI) and final weight gain (FWG) across the treatments with increase in *Jedi* inclusion. The mean weight gain (MWG) and food conversion ratio (FCR) were in the order of Diet 1> Diet 2> Diets 3 and 4> Diet 5 ($p< 0.05$). Similar trends of dominance of fish growth parameters in the control were observed in the protein intake (PI), specific growth rate (SGR) and relative weight gain (RWG) across all treatments with the highest values (13.8±0.33, 3.08±0.10 and 0.74±0.04 respectively) in the control diet and the least values (10.70±0.41, 2.52±0.01 and 0.52±0.07 respectively) in diet D.

Table 4: Growth parameters of *Clarias gariepinus* fed with graded levels of *Jedi*

Growth indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
IWG(g)	12.1±0.10	12.2±0.00	12.03±0.06	12.03±0.06	12.07±0.06
FWG(g)	$41.57±1.43^{a}$	$38.43±1.59^{b}$	$35.00±0.26^{c}$	$34.23±1.04^{d}$	$33.00±0.10^{e}$
MWG(g)	$29.47±1.53^{a}$	$26.30±1.71^{b}$	$22.97±0.23^{c}$	$22.20±0.98^{c}$	$20.93±0.06^{d}$
FI(g)	$39.43±0.93^{a}$	$36.63±1.18^{b}$	$35.37±0.99^{c}$	$33.33±1.20^{d}$	$30.57±1.16^{e}$
FCR	$29.47±1.53^{a}$	$26.30±1.71^{b}$	$22.97±0.23^{c}$	$22.20±0.98^{c}$	$20.93±0.06^{d}$
SGR(%/day)	$3.08±0.10^{a}$	$2.88±0.13^{b}$	$2.67±0.01^{b}$	$2.61±0.06^{b}$	$2.52±0.01^{b}$
PER(g)	$2.13±0.06^{a}$	$2.05±0.07^{a}$	$1.86±0.05^{b}$	$1.90±0.12^{b}$	$1.96±0.08^{b}$
RWG(g/day)	$0.74±0.04^{a}$	$0.66±0.04^{b}$	$0.57±0.01^{c}$	$0.56±0.02^{c}$	$0.52±0.07^{c}$
PI(g)	$13.8±0.33^{a}$	$12.82±0.41^{b}$	$12.38±0.35^{b}$	$11.67±0.42^{c}$	$10.70±0.41^{d}$

Values across the rows with different superscripts are significantly different ($p<0.05$).
FWG-final weight gain, IWG- initial weight gain, MWG-mean weight gain, FI-feed intake, FCR-feed conversion ratio, SGR-specific growth rate, PER-protein efficiency ratio, RWG-relative weight gain, PI-protein intake. Sample size (n)= 10.

Compared to Jedi, less growth variability occurred among the fish groups fed with varied *Gbewutu* treatments. No significant difference occurred in the FWG between the control and Diet 2 ($p>0.05$). The fish in control diet however, has significantly higher FWG, SGR, and RWG than the other treatments (Table 5). The MWG and FI of fish in the control were also significantly higher than other treatments. A steady decline in PI from control > Diet 2> Diet 3> Diet 4> Diet 5 occurred among the fish fed with *Gbewutu* ($p< 0.05$). No significant difference was observed in the IWG, FCR, and PER ($p> 0.05$).

Table 5: Growth parameters of *Clarias gariepinus* fed with graded levels of *Gbewutu*

Growth indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
IWG(g)	12.10±0.10	12.00±0.00	12.10±1.00	12.17±0.06	12.10±0.10
FWG(g)	43.03±1.79^{a}	40.23±0.61^{a}	36.57±1.60^{b}	34.60±1.20^{c}	31.73±1.19^{c}
MWG(g)	30.93±1.88^{a}	28.23±0.61^{b}	24.47±1.70^{c}	22.43±1.91^{d}	19.63±1.21^{d}
FI(g)	43.1±2.55^{a}	39.90±1.35^{b}	35.01±2.28^{c}	32.87±2.00^{c}	29.64±2.06^{d}
FCR(g)	1.39±0.03	1.41±0.06	1.44±0.14	1.47±0.04	1.51±0.09
SGR(g)	3.17±0.12^{a}	3.02±0.04^{a}	2.76±0.13^{b}	2.61±0.13^{b}	2.41±0.01^{b}
PER(g)	2.05±0.04	2.02±0.09	2.00±0.21	1.95±0.05	1.90±0.04
RWG(g)	0.77±0.05^{a}	0.71±0.02^{a}	0.61±0.04^{b}	0.56±0.05^{c}	0.49±0.03^{c}
PI(g)	15.09±0.89^{a}	13.97±0.47^{b}	12.25±0.80^{c}	11.50±0.70^{d}	10.37±0.72^{e}

Values across the rows with different superscripts are significantly different ($p<0.05$).
FWG - final weight gain, IWG - initial weight gain, MWG - mean weight gain, FI - feed FI- Feed intake, FCR - feed conversion ratio, SGR - specific growth rate, PI – protein intake, PER - protein efficiency ratio, RWG – relative weight gain. Sample size (n)= 10.

Compared to *Gbewutu*, the fish groups subjected to varied *Opa-eyin* treatments exhibited much less growth variations (Table 6). The MWG, FI, and PI of fish individuals in the control experiment were significantly higher ($p< 0.05$) than that of other treatments (among which no significant difference occurred). Result indicates that there was no concentration dependent growth impairement. No significant difference was observed among the SGR and PER of the control, Diet 2, and Diet 3, which were significantly higher than the other treatments. No significant difference also coccured among the FWG, MWG, and FI of fish given Diets 2, 3, and 4 ($p>0.05$); and the IWG across all treatments ($p>0.05$).

Notably, an unusual trend occurred in the case of FCR among fish fed with *Opa-eyin*. The FCR of fish fed with Diets 4 and 5 were significantly higher than those of Diets 2 and 3, which were higher than the control.

Table 6: Growth parameters of *Clarias gariepinus* fed with graded levels of *Opa-eyin*

Growth indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
IWG(g)	10.93±0.03	11.10±0.00	10.93±0.03	11.03±0.07	11.00±0.06
FWG(g)	30.07±1.88^{a}	26.73±1.04^{b}	25.77±1.48^{b}	21.63±1.00^{b}	20.33±0.70^{c}
MWG(g)	19.13±1.85^{a}	15.63±1.04^{b}	14.83±1.45^{b}	10.60±1.07^{b}	9.33±0.75^{b}
FI(g)	37.00±0.92^{a}	36.97±2.27^{b}	35.59±1.24^{b}	33.93±1.34^{b}	31.60±1.46^{b}
FCR(g)	1.96±0.13^{c}	2.37±0.12^{b}	2.44±0.21^{b}	3.62±0.22^{a}	3.37±0.08^{a}
SGR(g)	2.52±0.15^{a}	2.19±0.10^{a}	2.14±0.14^{a}	1.68±0.13^{b}	1.45±0.07^{b}
PER(g)	2.58±0.18^{a}	2.33±0.30^{a}	2.34±0.34^{a}	1.62±0.19^{b}	1.23±0.10^{c}
RWG(g)	174.90±16.36^{a}	140.84±9.38^{b}	135.61±12.91^{b}	96.07±10.32^{c}	84.84±7.73^{d}
PI(g)	7.40±0.18^{a}	6.85±0.61^{b}	6.43±0.33^{b}	6.37±0.16^{b}	6.11±0.28^{b}

NB: Values on the same row with different superscript are significantly ($P<0.05$) different from each other.
FWG- final weight, IWG- initial weight, MWG- mean weight gain, SGR- specific growth rate, RWG- relative weight gain, FI- feed intake, PI- protein intake, FCR- feed conversion rate, PER- protein efficiency ratio. Sample size (n)= 10.

The white blood cells (WBC) in the fish fed with Jedi was in the order of Deit 4> Diet 3> Diet 5> Diet 2> Control ($p< 0.05$). Meanwhile, the packed cell volume (PCV) was almost in the same order as WBC; in the order of Diet 4> Diet 3> Diet 5 and Diet 2> control ($p<0.05$). In similarity to WBC and PCV, Diets 3 and 4 were significantly higher in hemoglobin (Hb) and mean corpuscular hemoglobin (MCH) than Diets 5 and 2, which were in turn were higher than that of control ($p<0.05$). The red blood cells (RBC) in the fish fed with Diets 2, 3, and 4 were significantly higher than that of Diet 5 and control ($p<0.05$). The neutrophils (NEUT) in fish fed Diets 3

and 4 were significantly higher than Diet 2, which was higher than control> Diet 5> ($p<0.05$). The mean corpuscular volume (MCV) of fish in Diet 4> Diet 3 and 5> Diet 2> control ($p<0.05$). No significant difference occurred in the lymphocytes (LYPH), monocytes (MONO), platelets (PLT), and mean corpuscular hemoglobin concentration (MCHC) across the treatments and control ($p>0.05$).

Table 7: Hematological parameters of Juvenile *Clarias gariepinus* fed with graded levels of *Jedi.*

Hematological indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
WBC (cells x 103/µl)	20933.3 ± 208.17^{e}	23366.67 ± 182.01^{d}	25533.3 ± 104.1^{b}	26866.7 ± 121.5^{a}	24366.7 ± 121.5^{c}
PCV (%)	25.00 ± 1.00^{d}	27.00 ± 1.00^{b}	27.67 ± 0.58^{b}	29.33 ± 0.58^{a}	26.00 ± 1.00^{c}
Hb (g/dl)	11.30 ± 0.10^{c}	11.70 ± 0.10^{b}	11.93 ± 0.15^{a}	12.12 ± 0.25^{a}	11.60 ± 0.10^{b}
RBC (cells x 106/ µl)	0.93 ± 0.06^{b}	1.23 ± 0.12^{a}	1.30 ± 0.10^{a}	1.40 ± 0.10^{a}	1.17 ± 0.06^{b}
NEUT (%)	1.00 ± 0.00^{c}	1.33 ± 0.60^{b}	1.67 ± 0.60^{a}	2.00 ± 0.00^{a}	0.67 ± 0.60^{d}
LYPH (%)	99.0±0.00	99.33±0.60	99.67±0.60	100.00±0.00	98.67±1.15
MONO (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
PLT (cells x 103/µl)	97.33±0.60	99.00±1.00	100.00±1.00	100.33±1.15	96.67±5.77
MCHC (g/dl)	45.90±1.51	46.00±0.85	47.77±1.07	48.33±0.59	45.73±0.95
MCH (pg)	109.67 ± 2.08^{c}	113.00 ± 1.00^{b}	117.00 ± 1.00^{a}	120.33 ± 1.53^{a}	106.00 ± 1.00^{b}
MCV (fl)	252.00 ± 2.00^{d}	260.00 ± 2.00^{c}	262.00 ± 2.00^{b}	266.00 ± 0.00^{a}	264.00 ± 0.00^{b}

Values across the rows with different superscripts are significantly different ($p<0.05$).
WBC-white blood cells, PCV-packed cell volumes, Hb-hemoglobin, RBC-red blood cells, NEUT-neutrophil, LYPH-lymphocyte, MONO-monocyte, PLT-platelets, MCHC-mean corpuscular hemoglobin concentration, MCH-mean corpuscular hemoglobin, and MCV-mean corpuscular volume. Sample size (n)= 10.

The order of WBC in the fish fed with varied levels of *Gbewutu* was Diet 3, 4 and 5> Diest 2> control ($p< 0.05$). The effect of *Gbewutu* on the MONO was similar to that of WBC (Table 8). The order of MONO in the fish was Diet 3, 4 and 5> Diets 1 and 2 ($p<0.05$). The PCV in the control was however greater than those of Diets 2, 3, and 4> Diet 5 ($p<0.05$). *Gbewutu* had a slightly similar impact on the Hb of the fish. The Hb of the control was significantly higher than that of Diets 2 and 3, which was higher than Diets 4 and 5 ($p<0.05$). The MCH of the fish in the control experiment and Diet 4 were also higher than Diet 3> Diets 2 and 5 ($p<0.05$). Conversely, the MCV of Diets 2, 3 and 4> Diet 5> control ($p<0.05$). The herb had no significant effect on the RBC, NEUT, LYPH, PLT, and MCHC ($p> 0.05$).

Table 8: Hematological parameters of juvenile *Clarias gariepinus* fed with graded levels of *Gbewutu*

Hematological indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
WBC (x 103/µl)	20933.33 ± 208.17^{c}	23366.67 ± 182.0^{b}	26333.33 ± 100.67^{a}	26866.67 ± 194.51^{a}	26133.33 ± 202.67^{a}
PCV (%)	28.71 ± 1.00^{a}	27.65 ± 1.53^{b}	27.0 ± 2.00^{b}	26.67 ± 0.58^{b}	25.33 ± 0.58^{c}
Hb (g/dl)	13.60 ± 0.10^{a}	12.83 ± 0.15^{b}	12.10 ± 0.15^{b}	11.97 ± 0.12^{c}	11.63 ± 0.21^{c}
RBC (x 106/ µl)	1.70±0.00	1.64±0.06	1.51±0.00	1.32±0.06	1.30±0.06
NEUT (%)	0.33±0.58	0.67±0.58	1.00±0.00	1.03±0.06	0.37±0.58
LYPH (%)	98.00±0.00	98.67±0.58	99.33±0.58	100.00±0.00	98.33±0.58
MONO (%)	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.33 ± 0.58^{a}	0.33 ± 0.58^{a}	0.33 ± 0.58^{a}
PLT (x 103/µl)	102.00±1.00	102.33±1.53	102.33±1.15	102.67±0.58	102.33±0.58
MCHC (g/dl)	45.27±1.10	46.74±1.33	47.37±1.36	48.95±1.12	46.1±0.83
MCH (pg)	102.00 ± 2.00^{a}	117.33 ± 2.51^{c}	124.33 ± 2.52^{b}	130.00 ± 2.00^{a}	111.67 ± 1.53^{c}
MCV (fl)	235.67 ± 8.08^{c}	253.33 ± 2.89^{a}	256.67 ± 2.89^{a}	260.33 ± 10.00^{a}	241.33 ± 3.21^{b}

Values across the rows with different superscripts are significantly different ($p<0.05$).
WBC - white blood cell, PCV - packed cell volume, Hb - hemoglobin, RBC - red blood cell, NEUT – neutrophile, LYPH - lymphocyte, MONO – monocyte, PLT – platelets, MCHC – mean corpuscular hemoglobin concentration, MCH –mean corpuscular hemoglobin, MCV mean corpuscular volume. Sample size (n)= 10.

Opa-eyin caused an increasing trend in the WBC and PCV of the fish with increasing inclusions; in the order of Diet 5> 4> 3> 2> 1 ($p<0.05$). The Hb, RBC, and MCHC in the fish fed with Diets 3, 4 and 5 were significantly higher than in Diets 1 and 2 ($p<0.05$). The NEUT also exhibited a somewhat similar trend: Diets 4 and 5>

Diets 2 and 3, which were greater than the levels in fish in the control experiment ($p<0.05$). Conversely, the PLT in the control and Diet 4 were significantly higher than Diets 3 and 5, which were higher than the levels in fish on Diet 2 ($p<0.05$). The levels of MCHC and MCH in fish on *Opa-eyin* Diet 2 and control were not significantly different ($p>0.05$). The levels were however significantly lower than those of the fish on higher diet inclusions of the herb ($p<0.05$). The MCV of fish in the control experiment was also significantly lower than those of the fish on the higher diet inclusions among which no distinct pattern relative to the administered dosage was discerned.

Table 9: Hematological parameters of juvenile *Clarias gariepinus* fed with graded levels of *Opa-eyin*

Hematological indices	Diet 1(control)	Diet 2	Diet 3	Diet 4	Diet 5
WBC (x 10^3/µl)	20933.00±120.19^{e}	23800.00±100.00^{d}	24367.00±405.52^{c}	26200.00±709.46^{b}	29933.00±145.30^{a}
PCV (%)	25.00±0.58^{e}	26.66±0.33^{d}	28.33±0.66^{c}	31.33±0.88^{b}	36.33±0.33^{a}
Hb (g/dl)	11.47±0.09^{b}	11.63±0.12^{b}	12.06±0.03^{a}	12.23±0.09^{a}	12.30±0.11^{a}
RBC (x 10^6/ µl)	0.93±0.03^{b}	0.93±0.07^{b}	1.17±0.09^{a}	1.33±0.03^{a}	1.43±0.03^{a}
NEUT (%)	0.00±0.00^{c}	0.67±0.33^{b}	0.67±0.33^{b}	0.93±0.33^{a}	1.00±0.00^{a}
LYPH (%)	99.00±0.00	99.00±0.58	99.00±0.58	99.00±0.33	99.00±0.00
MONO(%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
PLT cells (x 10^3/µl)	102.00±0.58^{a}	92.67±0.58^{c}	97.00±1.15^{b}	100.67±0.33^{a}	97.33±0.88^{b}
MCHC (g/dl)	45.27±0.63^{b}	48.40±1.20^{b}	49.90±0.36^{a}	53.27±0.17^{a}	53.80±1.04^{a}
MCH (pg)	102.33±0.88^{d}	124.33±1.45^{d}	121.33±0.88^{b}	93.66±0.33^{a}	112.33±1.20^{c}
MCV (fl)	235.673±2.75^{c}	251.33±0.67^{a}	241.67±0.88^{b}	234.00±1.52^{c}	242.67±1.45^{b}

Values across the rows with different superscripts are significantly different ($p<0.05$).
WBC-white blood cells, PCV-packed cell volumes, Hb-hemoglobin, RBC-red blood cells, NEUT-neutrophil, LYPH-lymphocyte, MONO-monocyte, PLT-platelets, MCHC-mean corpuscular hemoglobin concentration, MCH-mean corpuscular hemoglobin, and MCV-mean corpuscular volume. Sample size (n)= 10.

DISCUSSION

Herbs are generally considered less toxic than synthetic alternatives because they are natural (Philomena, 2011). In this study, the cumulative effects of varied concentrations of the herbal concentrations over 40 d period showed noteworthy trends in the growth and hematological indices. The toxicity bioassay and the LC_{50} of the various herbs determined were 1.875061, 2.477121 and 2.176091 for *Jedi*,*Gbewutuand* and *Opa–eyin* respectively. The differences in the LC_{50} of the herbs may be as a result of the differences in types and concentrations of the phytochemical constituents of the plant used, which is attributable to the age and parts of the plants used as well as a function of the differences in the genetic make-up between species of the plant, including those of the climatic condition and the soil profile upon which the plants were grown (Borokini and Ayodele, 2012).

Results of the growth indices suggests that the appetite of the fish fed on the *Jedi* diets might have reduced with progressive inclsuions. This was characterized by the reduction in feed intake, hence final weight gain across with increasing *Jedi* inclusions. The suggested unpalatability of *Jedi* herb was buttressed by the mean weight gain and food conversion ratio, which both reduced with increased inclusion of the herb. Similarly, the inference was supported by the protein intake, specific growth rate and relative weight gain, which were all highest in the control fish and lowest in the highest *Jedi* inclusion. Furthermore, mobilization of the white blood cells and neutrophils in response to the inclsuions suggests that the immune system of the fish might have been sentitized by the herb, indicating possible toxic reactions.

In similar manner to the effects of *Jedi*, *Gbewutu* also exhibited stimulation of the defense system of the fish. Reduction of hemoglobin with increase in the

inclusion of *Gbewutu* further suggests possible hemolytic tendencies of the herb. This was corroborated by the decline in protein intake relative to increase in the inclusion of *Gbewutu.* Although *Gbewutu* didn't impair the growth of the fish as much as *Jedi,* results however suggest that the former might have impaired the nutrition of the fish to some extent. *Opa-eyin* appeared to be the most promising among all 3 herbal concoctions as the herb didn't impair the growth of the fish, at least not on the basis of incremental inclusions. Notably, results indicate that the herb might have improved the feed conversion rate of the fish and this effect appreciated with increase in the herbal inclusion. Optimum result was observed in the fish groups placed on the diet which contained the highest inclusion of *Opa-eyin.*

The final body weight and growth rate higher in fish fed control diet relative to fish fed with experimental diets could be related to the unpalatable nature of the feed with the inclusion of *Jedi* and *Gbewutu* extracts. According to Panigrahi and Powel (1991), to achieve efficient growth rate, feed intake must correspondingly increase to meet up with the anticipated growth rate of the animal. Hence, reduced growth observed with the test diets could be as a result of decrease in voluntary feed intake due to the presence of phenolic compounds the herbs. Phenolics are found in large quantities in the plants, and their biological functions include antioxidant activity and most importantly defence against pathogens, hence their use in the control of human and animal pathogenic infections. Herb extracts are also rich in secondary metabolite compounds such as volatile oils, saponins, tannins, alkaloids, polypeptides and polysaccharides which could be main sources of natural antioxidant and antimicrobial compounds for possible inclusion in aquatic feeds production (Tajodini *et al*., 2014). The current findings conflict the results obtained in aquacultural herbal additives reported by Adeyemo (2014), who evaluated the utilization of Roselle – *Hibiscus sabdariffaon* growth performance and production economy of *Clarias gariepinus* at varied concentrations. In this study, though the control diet had the best FCR, SGR and PER values, there was no significant ($p<0.05$) differences in the experimental diets for FCR and SGR particularly among the fish placed on diets with *Jedi* and *Gbewutu* inclusions. The current study is also at varience with the observations of Lawal *et al.* (2017) on *Gossypium herbaceum*. They reported a significant improvement in FCR of the fish fed varied levels of the experimental diet.

Hematological characteristics help fish biologists to interpret physiological responses by fish and deviation from normal response may indicate a disturbance in the physiological process. It was observed in this study that inclusion of the herbal concoctions in the diet of fish significantly increased the WBC, PLT, MCHC, MCH and MCV of the fish fed *Jedi* and *Gbewutu.* Significant increase was also observed in the PCV, Hb and RBC. According to Sweilum (2006), the alteration of blood parameters can be seen as compensatory responses that improve the oxygen carrying capacity to maintain the gas exchange in gill lamellae, which can also be an indicator of a change in water-blood obstruction. Similar results were reported in *Rhamdia quelen*, after exposure to diclofenac (Ghelfi *et al.*, 2015) and in *C. gariepinus* exposed to praziquantel (Nwani et al., 2014).

The increase in RBC values across the treatments with inclusion of *Jedi* and *Gbewutu* extracts relative to the control groups might be due to better cellular immunity as reported by Aderolu *et al*. (2017). This corroborates the observation of Sahu *et al*. (2007) who reported that increased RBC count in *Labeo rohita* fingerlings fed with *Mangifera* indica, which was an indication of enhanced cellular immunity. Bello *et al*. (2014) experimented with varied levels of *Tetracarpidium conophorum*

leaf and *Allium cepa* bulb, which did not significantly affect the hematological parameters of the juvenile *Clarias gariepinus.*

This study indicates the presence of a dose dependent immune system stimulating effect in *Clarias gariepinus*, particularly those fed graded levels of *Jedi* and *Gbewutu* extract. This result is in line with the findings of Choudhury *et al*. (2005) who reported that there was an increase in the WBC count in *Labeo rohita* juveniles, treated with immune-stimulants such as levamisole and ascorbic acid. Similar, reactions occurred when garlic peel was fed to *Clarias gariepinus* (Thanikachalam *et al*., 2010).

CONCLUSION

Jedi and *Gbewutu* herbs possess toxic tendencies and might be counterproductive in animals and humans. The study suggests that *Opa-eyin* has promising therapeutic potentials and might be an effective feed inclusion.

DECLARATION OF INTEREST

Authors declare no conflict of interest

ETHICAL STATEMENT

All experimental procedures were conducted in conformance to standard scientific ethics.

REFERENCES

Abramowitz, M.; Muntner, P.; Coco, M.; Southern, W.; Lotwin, I.; Hostetter, H. H. and Melamed, M. L.(2010). Serum Alkaline Phosphatase and Phosphate and Risk of Mortality and Hospitalization. Clin J Am Soc Nephrol; 5(6): 1064–1071.

Aderolu, A.Z. and Akpabio, V.M. (2009). Growth and economic performance of *Clarias gariepinus* juvenile fed diets containing velvet bean, *Mucumapruriens* seed meal. *Afr. J. Aquat. Sci*., 34: 131-135.

Adeyemo, R. (2004). Self-Help Farmer Cooperatives' Management of Natural Resources for Sustainable development in Southwest Nigeria. Journal of Rural Cooperation, 32(1), 3-18.

Aderolu, A.Z.; Lawal, M.O.; Soyinka, O.O.; Adeleke, A.T. and Bello, M.D. (2017). Antimicrobial and antioxidant properties of African medicinal plants. *J. Coast Life Med.*, 5(1):16-21.

Aksenes, A. and Njaa, L. (1981). Determina tion of catalase activity in fish. *Comp. Biochem. Physiol*,69: 893-896.

Bancroft, J. (2002). Light Microscopy. In: Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 5th ed. Philadelphia: *Churchill Livingstone Publishers*,12: 43–62.

Bello, O. S.; Olaifa, F. E. and Emikpe, B. O. (2014). Haematological and Blood Biochemical Changes in African Catfish, Clarias gariepinus Fed Walnut (Tetracarpidium conophorum Mull Arg) Leaf and Onion (Allium cepa Linn) Bulb Supplemented Diets. American Journal of Experimental Agriculture 4(12): 1593-1603

Ben Ameur, W.; de Lapuente, J.; El Megdiche, Y.; Barhoumi, B.; Trabelsi, S.; Camps, L.; Serret, J.; López, D.; Gonzalez-Linares, J.; Driss, M.R. and Borràs, M. (2012). Oxidative stress, genotoxicity and histopathology biomarker responsesin mullet (*Mugil cephalus*) and sea bass (*Dicentrarchus labrax*) liver from Bizerte Lagoon (Tunisia). *Marine Pollution Bulletin,* 64(2):241-251.

Borokini, T. I. and Ayodele, A. E. (2012). Pollen morphology of Taccaleontopetaloides(L.)kuntze in Nigeria .African journal of Plant science 6(1): 43-47.

Burkil, H. M. (2004). The useful plants of west tropical Africa. First edition. Publisher Royal Botanic Gardens: Kew, Richmond, United Kingdom. pp. 25-28.

Brima, E. I. (2017). Physicochemical properties and the concentration of anions, major and trace elements in groundwater, treated drinking water and bottled drinking water in Najran area, KSA Appl Water Sci 7: 401.

Chakraborty, S. B. and Hancz, C. (2011).Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture,* 3: 103-119.

Chakrabarti, R.; Srivastava, P. K.; Verma, N. and Sharma, J. (2014). Effect of seeds of *Achyranthes aspera* on the immune responses and expression of some immune-related genes in carp *Catla catla*. *Fish Shellfish Immunol.,* 41: 64-69.

Choudhury, S.; Sree, A.; Mukherjee, S.C.; Pattnaik, P. and Bapuji, M. (2005). In vitro antibacterial activity of extracts of selected marine algae and mangroves against fish pathogens. *Asian Fisheries Science*, 18: 285–294.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International,*18(3): 403-414.

Silva, M. D. C.; dos Santos, M. V. F. ; Dubeux, J. C. B. ; Lira, M. D. A. ; de Melo, W. S. ; de Oliveira, T. N. and de Araujo, G. G. L. (2004). Evaluation of pasture recovery methods pastures of *Brachiaria* at the agreste region of Pernambuco State. 2. Nutritive value of forage. Rev. Bras. Zootec., 33 (6-2): 2007-2016

Donmas, C.T.; Watson, W. and Briggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromocine-sol green. *Clinica Chacto,* 31: 87-96.

Edeoga, H. O.; Okwu, D. E. and Mbaeble, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech. 2005: 4:685-688.

Edet, A. E.; Worden, R. H.; Mohammed, E. A. and Preston, M. R. (2011). Hydrogeochemical processes in a populated shallow Coastal Plain Sand Aquifer southeastern Nigeria Environmental Earth Sciences DOI 10.1007/s12665-011-1175-9. 21pp

Elujoba, T. (2005). Traditional medicine plants and malaria. Afr. J. Tradit. Complement. Altern. Med. 2(2):206-207.

Ernst, E. (2003). Cardiovascular adverse effects of herbal medicines: A systemic review of the recent literature. Can J Cardiol.; 19(7):818-827. 9.

Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plant used in traditional medicine for drug discovery. Environ Health Perspect; 109(10): 69-75.

Fasuyi, A.O., (2006). Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. Afr. J. *Biotech*., 5: 49-53.

Frankel, S.; Gunnel, D.J. and Peters, T.J. (1998). Childhood energy intake and adult mortality from cancer: the Boyd Orr Cohort Study. *British Medical Journal,* 316: 499-504.

Fricke, N.F.; Stentiford, G.D.; Feist, S.W. and Lang, T. (2012). Liver histopathology in Baltic eelpout (*Zoarces viviparus*). A baseline study for use in marine environmental monitoring. *Marine Environmental Research*,82: 1-14.

Ghasemzadeh, A.; Jaafar, H.Z. and Rahmat, A. (2010). Antioxidant activities total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*,15(6): 4324-4333.

Ghasemzadeh, A. and Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of Medicinal Plants Research*,5(31): 6697-6703.

Ghelfi, A.; Luiz, J.; Ribas, C.; Guiloski, I. C.; Bettim, F. L.; Piancini, L. D. S.; Cestari, M. S.; Pereira, A. J.; Sassaki, G. L. and Silva de Assis, H., C. (2015). Evaluation of Biochemical, Genetic and Hematological Biomarkers in a Commercial Catfish Rhamdia quelen Exposed to Diclofenac. Bull Environ Contam Toxicol (2016) 96:49–54

Gill, L.S. (1992). Ethnomedical uses of plants in Nigeria, 1st edition, University of Benin press, Benin city. Pp 180-181.

Gonall, A.G.; Bardawill, C.J. and David, M.M. (1949).Determination of total protein.*Journal of Biological Chemistry*, 177: 751-760.

Govind, P. (2012). Medicinal plants against liver diseases. *International Research Journal of Pharmacy*, 2: 115–121.

Gu, Z.; Aimetti, A.A.; Wang, Q.; Dang, T.T.; Zhang, Y.; Veiseh, O.; Cheng, H.; Langer, R.S. and Anderson, D.G. (2013)*ACS Nano*, 7: 4194-4201.

Haghighi, M. and Sharifrohani, M. (2013). The effects of powdered ginger rhizome (*Zingiber officinale*) on haematological and immunological parameters of rainbow trout *Oncorhynchus mykiss*. *Asian Journal of Marine Science*s, 1: 7-11.

Harikrishnan, R.; Kim, J.S.; Kim, M.C.; Balasundaram, C. and Heo, M.S. (2011). Kalopanax pictus as feed additive controls bacterial and parasitic infections in kelp grouper, *Epinephelus bruneus*. *Fish Shellfish Immunol.*,31:801–807.

Harikrishnan, R.; Kim, J.S.; Balasundaram, C. and Heo, M.S. (2012). Dietary supplementation with chitin and chitosan on haematology and innate immune response in Epinephelus bruneus against Philasterides dicentrarchi. *Exp. Parasitol.*, 131: 116–124.

International Committee for Standardization in Hematology (ICSH) (1967). Recommendations for hemoglobinometry in human blood. *Br J Haematol.* 13: 71-76.

International Committee for Standardization in Hematology (ICSH) (1996). Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for internationsl haemiglobinocyanide standard. 4th Edition. J Clin Path. 49: 271- 274.

John, F. N. (2001). Ancient Egyptian medicine. University of Oklahoma Press (USA). 151.

Johnson, C. and Banerji, A. (2007). Influence of Extract Isolated from the Plant Sesuvium *portulacastrum* on Growth and Metabolism in Freshwater Teleost, *Labeo rohita* (Rohu). *Fishery Tech.*, 44(2): 229-234.

Kalaivani, T. and Mathew L. (2010). Free radical scavenging activity from leaves of Acacia nilotica (L.) Wil. ex Delile, an Indian medicinal tree. *Food Chem. Toxicol.*; 48:298-305

Kelly, J.M. and Janz, D.M. (2009). Assessment of oxidative stress and histopathology in juvenile northern pike (*Esox lucius*) inhabiting lakes downstream of a uranium mill. *Aquatic Toxicology*, 92(4):240-249.

Lamidi, M.; Oliver, E.; Faure, R.; Debrauwer, L.; Nze-Ekekang, L. and Balansard, G. (1995). Quinovic acid glycoside from *Nauclea diderichii. Planta Med.* 61:280-281

Lans, C.A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J. Ethnobiol Ethnomed*,2: 45-55.

Lawal, T.O.; Bamiduro, T. B.; Ofonmbuk, J. M.; Elufioye, T.O.; Adeniyi, B. A. and Mahady, G. B. (2017). Antibacterial effects of Anogeissus leiocarpus (DC.) Guill. & Perr. and Terminalia glaucescens Planch. Ex Benth. on rapidly growing mycobacteria species African Journal of Microbiology Research 11(12): 495-503

Mensah, J.K.; Okoli, R. I.; Ohaju-Obodo, J.O. and Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. Afr. J. Biotechnol., 7: 2304-2309.

Moraes, T.F.; Edwards, R.A.; McKenna, S.; Pastushok, L; Xiao, W.; Glover, J.N. and Ellison, MJ. (2001). Crystal structure of the human ubiquitin conjugating enzyme complex, hMms2-hUbc13. *Nat Struct Biol* 8(8):669-73

Neuwinger, H. D. (1996). African Ethnobony. Poisons and Drugs. Chapman and Hall, Wienheim

Neuwinger, H. D. (2000). African Traditional Medicine. A Dictionary of Plant Use and Applications. Medpharm Scientific Publishers, Stuttgard, Germany. ISBN 3–88763–086–6

Nwani, C.D.; Nnaji, M.C.; Oluah, S.N.; Echi, P.C.; Nwamba, H.O.; Ikwuagwu, O.E. and Ajima, M.N.O. (2014). Mutagenic and physiological responses in the juveniles of African catfish, *Clarias gariepinus* (Burchell 1822) following short-term exposure to praziquantel. *Tissue and Cell* 46: 264–273

Oreagba, I. A.; Oshikoya, K. A. and Amachree, M. (2011). Herbal medicine use among urban residents in Lagos, Nigeria. *BMC Complemetary and Alternative Medicine* 11:117

Panigrahi, S. and Powell, C. J. (1991). Effects of high rates of inclusion of palm kernel meal in broiler chick diets. Anim. Feed Sci. Technol., 34: 37-47.

Rosechlau, P.; Bernt, E. and Gruber, J.W. (1974). Serum determination of cholesterol and quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. Clin Biochem., 12: 403-407.

Sahu, S.; Das, B. K.; Pradhan, J.; Mohapatra, B. C.; Mishra, B. K. and Sarangi, A. (2007). Effect of Magnifera indica kernel as a feed additive on immunity and resistance to Aeromonas hydrophila in Labeo rohita fingerlings. Fish and Shellfish Immunology, 23: 109-118.

Sedlak, J. and Lindsay, R.H. (1968). Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's rea gent. *Anal Biochem.,* 25: 1192-1205.

Senthil Kumar, M.; Kumar, G.; SrikanthBabu, V. and Udayakumar, M. (2006). Assessment of Variability in Acquired Tolerance: Potential Option to Study Genotypic Responses and the Relevance of Stress Genes. *J. Plant Physiol.* 164(2):111-125

Senthilkumar, R.; Rajkapoor, B.; Perumal, P.; Dhanasekaran, T.; Alvin Jose, M. and Jothimanivannan, C. (2009). Anti-inflammatory and analgesic activities of ethanol extract of Indigofera trita linn. Pharmacologyonline., 1: 278-289

Solomon-Wisdom, G. O. and Shittu, G. A. (2010). In vitro antimicrobial and phytochemical activities of Acacia nilotica leaf extract. Journal of Medicinal Plants Research 4(12): 1232-1234

Somjetlerdcharoen, A. (2002). Chloramphenicol concerns in shrimp culture. Aquaculture Asia, 2002, 7, 51-4.

Sun, M. and Zigma, S. (1978). An improved Spectrophotometer assay of Superoxide Dismutase Based on Epinephrine Antioxidation. *Analytic Biochemistry*, 90: 81-89.

Sweilum, M. A. (2006). Effects of sublethal toxicity of some pesticides on growth parameters hematological haematological properties and total production of Nile tilapia (*Oreochromis niloticus* L.) and water quality of ponds *Aquatic Research* 37(11): 1079-1089

Syahidah, A.; Saad, C.; Daud, H. and Abdelhadi, Y. (2015). Status and potential of herbal applications in aquaculture: A review. I*ranian Journal of Fisheries Sciences*,14(1): 27-44.

Tajodini, M.; Moghbe, P.; Saeedi, H. R. and Effati, M. (2014). The Effect of Medicinal Plants as a Feed Additive in Ruminant Nutrition. Iranian J. Appl. Anim. Sci. 4(4): 681-686.

Thanikachalam, K.; Kasi, M. and Rathinam, X. (2010). Effect of garlic peel on growth, hematological parameters and disease resistance against Aeromonas hydrophila in African catfish Clarias gariepinus (Bloch) fingerlings. Asian Pac. J. Trop. Med. 3 (8), 614–618.

Tovar, R. T. and Petzel, R. M. (2009). Herbal Toxicity. Dis Mon. 55(10):592-641.

Turan, F. and Akyurt, I. (2005). Effects of red clover extract on growth performance and body composition of African catfish (*Clarias gariepinus*). *Fisheries Sciences*, 78: 618- 620.

Usman, H.; Yaro, A. H. and Garba, M. M. (2008). Phytochemical Anticonvulsant Screening of the Ethanolic Flower Extracts of *Newbouldia laevis* (Bignoniaccae) in Mice. *Journal Pharmacology and Toxicology* 3(2):127-133

Van Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: A review. *Aquaculture*, 446: 88-96.

Van Kampen, E. J. and Zijlstra, W. J. (1961). Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clin Chim Acta.* 6: 538- 544.

Vanucci, C.; Lange, C.; Lhommet, G.; Dupont, B.; Davoust, D.; Vauchot, B.; Clement, J. L. and Brunck,F. (1992) .Aninsectantifeedantlimonoidfromseedof Khayaivorensis. Phytochemistry31,3003–3004.

Vermal, G.; Dua, V.K.; Agarwal, D.D. and Atul PK. (2011). Antibacterial activity of Holarrhena antidysenterica and Viola canescens plants traditionally used against malaria in the Garhwal region of north-west Himalaya. *Malar J.*;10:20.

Wu, C.C.; Liu, C.H.; Chang, Y.P. and Hsieh, S.L. (2010). Effects of hot-water extract of *Toona sinensis* on immune response and resistance to *Aeromonas hydrophila* in *Oreochromis mossambicus*. *Fish and Shellfish Immunology*, 29: 258-263.

Yakubu, M. T. and Jimoh, R. O. (2015) Aqueous extract of Corpolobia lutea roots ameliorates paroxetine-induced anti-androgenic activity in male rats. Middle East Fertility Journal 20: 192-197.

Yemitan, O.K. and Adeyemi, O. O. (2004). Toxicity studies of the aqueous root extract of *Lecaniodiscus cupanioides*. Nig. J. Health Biomed. Sci., 3: 20-23.