



## Growth promoting and anti-microbial potentials of *Spondias mombin* L. and *Amaranthus viridis* L. in *Clarias gariepinus* (Burchell, 1822) (African sharptooth catfish) production.

OYEBANJI BUKOLA OLANIKE\*, MAKINDE TAIWO AND AGBOLUAJE BAMIDELE

Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

\*Corresponding author email: [oyebanji.bukola44@gmail.com](mailto:oyebanji.bukola44@gmail.com)

### Abstract

This study was conducted to evaluate the effect of dietary inclusion of *Amaranthus viridis* (AV) and *Spondias mombin* (SM) leaf extract on growth, microbial load and haematological parameters of *Clarias gariepinus* (Burchell, 1822). One hundred and twenty fingerlings were randomly distributed into plastic tanks (45 L) at 10 fish /tank and replicated twice. Group 1 animals served as control, groups 2 and 3 had 250 mg/kg and 500 mg/kg of AV, groups 4 and 5 had 250 and 500 mg of SM, respectively, group 6 animals had 500 mg/kg of vitamin C. The weight of the animals were recorded weekly while hematological parameters were determined and visceral weight measured at the end of the experiment. The mean weight gain, final standard length and the viscerotropic indices were significantly different ( $p < 0.05$ ) (ANOVA,  $n=9$ ) across the group with the highest values recorded in SM250 mg/kg group. Total microbial load was least in the AV500 group ( $42.5 \pm 12.38$ ). Fish in AV250 group had the highest packed cell volume and white blood cell values of fish across the group. It can be concluded that the *Spondias mombin* has growth enhancing potential while *Amaranthus viridis* has immunostimulatory and antimicrobial potential and hence both can find usefulness in African catfish production.

**Keywords:** *Amaranthus viridis*, Antimicrobial, Growth, *Spondias mombin*

### Introduction

The African Catfish (*Clarias gariepinus*, Burchell, 1822) culture is widely practiced in many tropical and subtropical regions of the world and constitutes one of the largest groups of farmed freshwater fish (Adedeji and Adegbile, 2011). Catfish farming and indeed aquaculture offer strong potential for growth to meet the natural fish demand

thereby reducing importation, provides employment, alleviates poverty and helps to meet the millennium development goals (Williams *et al.*, 2007). The quality and quantity of food produced either from land or water is inadequate with teeming population which appears to be doubling every 35 years (Bell and Counterbery, 2000).

The recent expansion of intensive aquaculture practices has led to high interest in understanding the various fish diseases, so that they can be treated or prevented (Kaleeswaran *et al.*, 2012). According to Kaleeswaran *et al.* (2012) and Bernard *et al.* (2015), it is widely demonstrated that the occurrence of diseases in fish farm is due to several factors concerned with the rearing methods, environmental conditions and variations. Consequently, cultivated fish can become more susceptible not only to pathogenic but also to opportunistic bacteria (Woo and Bruno, 1998). In intensive aquaculture, the use of antibiotics and chemotherapeutics for treatment and prophylaxis has been broadly criticized for its negative impact (FAO, 2002). Research on interactions between growth, immunity and development of eco-friendly alternatives to antibiotics that may keep fish healthy is being encouraged. The use of materials such as probiotics and plant based immunostimulants has increased, indigenous technological knowledge for treating diseases is enjoying attention in fish health and disease management (Sahu *et al.*, 2007a&b).

There has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been assessed (Denev, 2008). A plant becomes a medicinal plant only when its biological activity has been ethno botanically reported or scientifically established (Elujoba, 1997).

Many plant-compounds have been found to have non-specific immuno-stimulating effects in humans and animals (Pandey and Madhuri, 2010; Kolkovski and Kolkovski, 2011) of which more than a dozen have been evaluated in fish and shrimp. Several plant products seemed to be potent antiviral

agents against fish and shrimp viruses (Kolkovski and Kolkovski, 2011). *Azadirachta indica* (Neem) tree have been studied by Chitmanat *et al.* (2005) for its insecticidal and antiviral properties. Indian almond (*Terminalia catappa*) and garlic (*Allium sativum*) have been said as an alternative to chemicals to treat fish ectoparasites, *Trichodina sp.* infections in Tilapia (*O. niloticus*) fingerlings. The immunostimulant effects of the dietary intake of 3 plants (*Viscum album*, *Urtica dioica* and *Zingiber officinale*) extracts on Rainbow trout (*Oncorhynchus mykiss*) have also been demonstrated (Chitmanat *et al.*, 2005). Christyapita *et al.* (2007) observed the immunostimulatory effect of aqueous extract of *Eclipta alba* (Bhangra) leaf (oral administration as feed supplement) in tilapia fish, *Oreochromis mossambicus*.

*Amaranthus viridis* (Amaranthaceae), also called green amaranth commonly known as “tete abalaye” in Yoruba, is a fast growing herb found in Nigeria. This pseudo cereal has attracted much attention as an important food commodity (Saxena *et al.*, 2007). The leaves are diuretic and purgative, and are used in poultices (fresh or as dried powder) to treat inflammations, boils and abscesses, gonorrhoea, orchitis and haemorrhoids. In Nigeria an infusion of the whole plant is used to purify the blood and the pounded root is applied against dysentery. In Côte d’Ivoire leaf sap is used as an eye wash to treat eye infections and for treating convulsions and epilepsy in children (Brenan, 1981; Burkill, 1985). The antioxidant properties of green leafy vegetables and herbs including different Amaranth species have been preliminarily studied (Ozbucak *et al.*, 2007). Isolation of the antifungal peptide from the *A. viridis* seed extracts has been done (Lipkin *et al.*, 2004) and the *in vitro* activities of the leaf and seed extract against some food-borne

and pathogenic bacteria confirmed (Muhammad *et al.*, 2012).

*Spondias mombin* or sour fruit is a fructiferous tree having habitat its Nigeria, Brazil and several other tropical forests in the world (Akubue *et al.*, 1983). This plant is readily common around us in South West of Nigeria and is commonly used in human medicine. Various cultures frequently maintain within their collection of traditional medicine substances valued as drugs for treating diseases (Elizabethshy *et al.*, 1992). The fruit juice is drunk as a diuretic and febrifuge, the decoction of the astringent bark serves as an emetic, a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leukorrhoea. In Mexico, it is believed to expel calcifications from the bladder, the powdered bark is applied to treat wounds. A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, urethritis, cystitis and eye and throat inflammations (Ayoka *et al.*, 2008). *Spondias mombin* as a medicinal plant with a lot of potential, valuable, untapped resource of active drugs for treating diseases (Ayoka *et al.*, 2008).

This present study was conducted to determine the potential of the methanolic extract of *A. viridis* and *S. mombin* on growth, haematology and total microbial count of African catfish (*Clarias gariepinus*, Burchell 1822), with the hypothesis: *A. Viridis* and *S. mombin* have immunostimulatory and antibacterial properties that can enhance growth and health of *C. gariepinus*.

### Materials and Methods

The experiment was conducted at the Faculty of Agriculture, Department of Animal Sciences Wet laboratory, Obafemi Awolowo University Ile-Ife Osun State located in the South West Nigeria. Fingerlings of *C. gariepinus* were bought

from the Wet Laboratory of Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife and acclimatized to experimental condition for 14 days at room temperature prior to the feeding trial.

### Preparation of Plant Extract and Fish Feed

Fresh leaves of *A. viridis* and *S. mombin* were harvested from the Obafemi Awolowo University surroundings. They were authenticated at the herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife where voucher specimen was deposited. The plants were air dried and ground to powder forms after drying using a burr mill. Powdered and weighed samples of the leaves were extracted in 100% methanol by soaking for 72 hours (Abah and Egwari, 2011). The resulting crude methanolic extracts was then concentrated under reduced pressure at 35°C in a rotary evaporator to obtain the solid samples which was weighed and stored in the dessicator for pharmacological studies. The extracts were weighed with sensitive weighing scale (Mettler Toledo, AB54, Germany) and mixed with olive oil after which the mixture was mixed with the fish diet. Thereafter, the diet was air dried and assigned to an experimental treatment. This method is known as surface coating (Adeparusi and Famurewa, 2011).

### Experimental setup and feeding protocol

At the end of the acclimatization period, a total of 120 fingerlings (17.5 ±0.5g) were divided into six treatment of 10 fish each and replicated (R) twice.

T1-----Control

T2-----Vitamin C 500 mg/kg

T3-----*Amaranthus viridis* 250mg/kg

T4-----*Amaranthus viridis* 500mg/kg

T5-----*Spondias mombin* 250mg/kg

T6-----*Spondias mombin* 500mg/kg

The fish were kept in 45L rectangular plastic tanks. Each culture treatment was fed with durante (2mm) floating diet, a complete dry catfish food containing 45% of protein. Each experimental diet was randomly assigned to duplicate tanks. The fingerlings were fed 5% of their body weight with the respective diet twice daily, morning between 08:00 and 10:00 hours and evening between 16:00 pm and 18:00 hours. During the trial, the water temperature was maintained at  $29.0 \pm 2.0^\circ\text{C}$  and Dissolved oxygen ranged from 3.10-6.13 mgL<sup>-1</sup>. The experimental unit was under a natural light and dark cycle.

#### **Data collection**

The weighing and length measurement of fish were carried out on weekly basis with Mettler weighing scale and tape rule respectively. On weighing days, the fishes were not fed in the morning until the whole exercise was completed and fed in the late afternoon. The feeding trials lasted for six weeks (42 days). The use of the animal was approved by the approved by the Department of Animal Sciences Ethical committee of Obafemi Awolowo University, Ile-Ife.

At the end of the six-weeks trial, the final body (FBW), Weight mean (WG) mean weight gain (MWG), feed conversion rate (FCR), percentage survival rate (%), condition factor (CF), were measured and calculated as follows;

**Weight gain (WG, g)** = (Final weight – Initial weight).

**Mean weight gain (MWG, g)** = (Final mean weight – Initial mean weight).

**Survival rate (SR, %)** = The survival rate was calculated as total number of fish harvested/total number of fish stocked expressed in percentage.

**Food conversion rate (FCR)** = (Total feed consumed by fish divided by Weight gain by fish).

**Condition factor (CF):**  $K = w * 100/L^3$   
(Where w = weight of fish in (g), L = length of fish in (cm)).

Blood samples were collected from five fishes per tank. It was collected by puncturing the caudal veins of fish and examined for the haematological parameters. Standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (Hb) concentration was estimated as cyanmethemoglobin (Brown, 1980) where as Packed Cell Volume (PCV) was determined using microhaematocrit method as adopted from Snieszko (1960). The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe (1978). Also the total White Blood Cell Counts (WBC) was enumerated with an improved Neubauer Haemocytometer using Shaw's diluting fluid (Miale, 1982). The Erythrocyte Sedimentation Rate (ESR) was done by Wintrobe method (Wedemeyer *et al.*, 1983). Thrombocyte count was performed according to Rees and Ecker method (Seiverd, 1983). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated respectively using standard formula described by Barbara *et al.* (2012) and Joshi *et al.* (2002).

#### **Viscero, Hepato, and Spleno somatic Indices**

The 10 sacrificed fishes from each treatment were dissected. The visceral, liver and spleen were removed, weighed and viscerosomatic, hepatosomatic and spleno somatic indices (VSI, HIS and SSI) were calculated as follows;

**HSI (%)** =  $100 \times [\text{liver weight (g)}] / \text{whole fish weight (g)}$ .

**VSI (%)** = 100 x [viscera weight (g)]/whole fish weight (g).

**SSI (%)** = 100 x [spleen weight (g)]/whole fish weight (g).

### Statistical Analysis

Data obtained were subjected to one way Analysis of Variance [ANOVA (Zar, 1999)] and the mean were separated using Duncan multiple range test. SAS package (Version 17.1) was used in data analysis. Significant differences were considered at the significance level of .05. The results were expressed as mean  $\pm$  SEM (Standard error of mean).

### Results

*C. gariepinus* administered with *A. viridis* at 250 mg/kg showed significantly higher value for Packed cell volume (PCV), followed by those treated with *A. viridis* at 500 mg/kg and Vitamin C. The lowest value for PCV was obtained from fish in the control group and *S. mombin* 500 mg/kg group. The haemoglobin (Hb) values, RBC and WBC obtained from *C. gariepinus* treated with *A. viridis* at 250 mg/kg increased significantly followed by those treated with *A. viridis* at 500 mg/kg. Highest platelet number was recorded from *C. gariepinus* fish treated with *A. viridis* 250 mg/kg (Table 1).

**Table 1:** Effect of *Spondia mombin* and *Amaranthus viridis* on haematological parameters of *Clarias gariepinus* fingerlings

	CONTROL	VITAMIN C	AV250	AV500	SM250	SM500
PCV (%)	15.00 $\pm$ 1.41 <sup>a</sup>	22.10 $\pm$ 1.41 <sup>ab</sup>	31.70 $\pm$ 1.41 <sup>d</sup>	26.80 $\pm$ 1.41 <sup>c</sup>	21.30 $\pm$ 1.41 <sup>b</sup>	18.90 $\pm$ 1.41 <sup>ab</sup>
Hb (g/dl)	4.95 $\pm$ 0.48 <sup>a</sup>	7.31 $\pm$ 0.48 <sup>b</sup>	10.49 $\pm$ 0.48 <sup>d</sup>	8.86 $\pm$ 0.48 <sup>c</sup>	7.04 $\pm$ 0.48 <sup>b</sup>	6.21 $\pm$ 0.48 <sup>ab</sup>
RBC( $\times 10^6$ )	2.21 $\pm$ 0.45 <sup>a</sup>	1.67 $\pm$ 0.45 <sup>a</sup>	5.13 $\pm$ 0.45 <sup>b</sup>	2.24 $\pm$ 0.45 <sup>a</sup>	1.93 $\pm$ 0.45 <sup>a</sup>	1.64 $\pm$ 0.45 <sup>a</sup>
WBC( $\times 10^3$ )	5.06 $\pm$ 0.53 <sup>a</sup>	3.75 $\pm$ 0.53 <sup>a</sup>	7.02 $\pm$ 0.53 <sup>b</sup>	4.07 $\pm$ 0.53 <sup>a</sup>	3.87 $\pm$ 0.53 <sup>a</sup>	4.26 $\pm$ 0.53 <sup>a</sup>
Thromb ( $\times 10^3/\text{mm}^3$ )	3.50 $\pm$ 0.48 <sup>a</sup>	6.50 $\pm$ 0.48 <sup>c</sup>	9.00 $\pm$ 0.48 <sup>d</sup>	8.00 $\pm$ 0.48 <sup>d</sup>	6.00 $\pm$ 0.48 <sup>c</sup>	5.00 $\pm$ 0.48 <sup>b</sup>
LYM (%)	68.80 $\pm$ 2.28 <sup>ab</sup>	69.00 $\pm$ 2.28 <sup>ab</sup>	72.60 $\pm$ 2.28 <sup>b</sup>	64.20 $\pm$ 2.82 <sup>a</sup>	61.90 $\pm$ 2.28 <sup>a</sup>	63.40 $\pm$ 2.28 <sup>a</sup>
NEUT (%)	29.90 $\pm$ 2.29 <sup>ab</sup>	30.00 $\pm$ 2.29 <sup>ab</sup>	26.80 $\pm$ 2.29 <sup>a</sup>	34.80 $\pm$ 2.29 <sup>b</sup>	37.00 $\pm$ 2.29 <sup>b</sup>	35.40 $\pm$ 2.29 <sup>b</sup>
Monocyte (%)	1.30 $\pm$ 0.12 <sup>b</sup>	1.00 $\pm$ 0.12 <sup>b</sup>	0.50 $\pm$ 0.12 <sup>a</sup>	1.00 $\pm$ 0.12 <sup>b</sup>	1.10 $\pm$ 0.12 <sup>b</sup>	1.20 $\pm$ 0.12 <sup>b</sup>
MCH (pg)	22.40 $\pm$ 4.66 <sup>a</sup>	43.77 $\pm$ 4.66 <sup>c</sup>	20.44 $\pm$ 4.66 <sup>ab</sup>	47.00 $\pm$ 4.66 <sup>c</sup>	39.55 $\pm$ 4.66 <sup>bc</sup>	37.86 $\pm$ 4.66 <sup>c</sup>
MCV (fl)	67.87 $\pm$ 14.32 <sup>a</sup>	132.33 $\pm$ 14.32 <sup>d</sup>	61.79 $\pm$ 14.32 <sup>c</sup>	130.50 $\pm$ 14.32 <sup>c</sup>	119.64 $\pm$ 14.32 <sup>bc</sup>	115.24 $\pm$ 14.32 <sup>ab</sup>
MCHC (pg)	33.00 $\pm$ 0.00 <sup>a</sup>	33.00 $\pm$ 0.00 <sup>a</sup>	33.00 $\pm$ 0.00 <sup>a</sup>	33.00 $\pm$ 0.00 <sup>a</sup>	33.00 $\pm$ 0.00 <sup>a</sup>	33.00 $\pm$ 0.00 <sup>a</sup>

Means having different superscript in the same row are significantly different at  $p < 0.05$ .

**Keys:** PCV=Pack cell volume, HB=Haemoglobin, RBC=Red blood cell, WBC=White blood cell, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration, LYM=Lymphocyte, NEUT=Neutrophil.

The highest body weight gain was recorded in fish administered with *S. mombin* 250mg/kg (Table 2). There was no significant difference in weight of spleen, hepatosomatic index and splenosomatic

index across the group (Table 3). *C. gariepinus* fish treated with *A. viridis* showed the lowest total microbial count (Table 4).

**Table 2:** Effect of *Spondia mombin* and *Amaranthus viridis* on growth performance of *Clarias gariepinus* fingerlings

Parameters	CONTROL	VITAMIN C	AV250	AV500	SM250	SM500
INITIAL WT.(g)	17.5±0.00	17.5±0.00	17.5±0.00	17.5±0.00	17.5±0.00	17.5±0.00
FINAL WT.(g)	38.05±1.82 <sup>b</sup>	36.70±1.82 <sup>ab</sup>	37.20±1.82 <sup>ab</sup>	30.90±1.82 <sup>a</sup>	42.10±1.82 <sup>b</sup>	36.30±1.82 <sup>ab</sup>
Mean Wt gain	20.55±1.82 <sup>b</sup>	19.20±1.82 <sup>a</sup>	19.70±1.82 <sup>ab</sup>	13.40±1.82 <sup>a</sup>	24.60±1.82 <sup>b</sup>	18.80±1.82 <sup>ab</sup>
Length (cm)	18.80±1.57 <sup>b</sup>	16.47±0.96 <sup>ab</sup>	17.66±2.33 <sup>ab</sup>	16.45±1.32 <sup>a</sup>	18.74±1.52 <sup>b</sup>	17.43±1.83 <sup>ab</sup>
% Weight gain	116.57	96.57	112.57	76.57	140.57	121.71
FCR	2.02	2.32	2.07	2.77	1.81	1.98
% SURVIVAL	100	100	100	85	100	100

abcd = Means with different superscript in a row are significantly different at  $p < 0.05$ .

**Table 3:** Effect of *Spondia mombin* and *Amaranthus viridis* on organs of *Clarias gariepinus* fingerlings

Parameter	CONTROL	VITAMIN C	AV250	AV500	SM250	SM500
Visceral	0.98±0.01 <sup>ab</sup>	0.71±0.07 <sup>a</sup>	1.23±0.69 <sup>bc</sup>	0.85±0.19 <sup>ab</sup>	1.40±0.28 <sup>c</sup>	1.16±0.43 <sup>b</sup>
Liver	0.33±0.02 <sup>ab</sup>	0.30±0.08 <sup>ab</sup>	0.36±0.09 <sup>b</sup>	0.23±0.07 <sup>a</sup>	0.33±0.01 <sup>b</sup>	0.30±0.08 <sup>ab</sup>
Spleen	0.03±0.01	0.04±0.01	0.04±0.02	0.03±0.01	0.04±0.01	0.03±0.01
HSI	8.82±0.74	8.67±2.36	9.64±2.74	8.03±2.06	7.82±2.74	7.83±2.06
SSI	0.86±0.19	1.02±0.22	1.05±0.72	1.26±0.46	1.09±0.16	0.96±0.33
VSI	25.95±0.50 <sup>ab</sup>	20.39±2.17 <sup>a</sup>	27.44±7.02 <sup>bc</sup>	27.57±6.38 <sup>bc</sup>	33.30±6.99 <sup>c</sup>	29.85±10.80 <sup>bc</sup>

abcd = Means with different superscript in a row are significantly different at  $p < 0.05$ .

**Table 4:** Effect of *Spondia mombin* and *Amaranthus viridis* on total microbial count of *Clarias gariepinus* fingerlings

Treatment	Total Microbial Load (CFU/ml) 10 <sup>4</sup>
Control	199.50 ± 12.38 <sup>d</sup>
Vitamin C 500 mg/kg	154.00 ± 12.38 <sup>c</sup>
<i>A. viridis</i> 250 mg/kg	119.00 ± 12.38 <sup>b</sup>
<i>A. viridis</i> 500 mg/kg	42.50 ± 12.38 <sup>a</sup>
<i>S. mombin</i> 250 mg/kg	128.00 ± 12.38 <sup>bc</sup>
<i>S. mombin</i> 500 mg/kg	103.00 ± 12.38 <sup>b</sup>

abcd = Means with different superscript are significantly different at  $p < 0.05$ .

## Discussion

The studies of blood parameters had proven to be a valuable approach for analyzing the health status of fish and help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment (Bahmami *et al.*, 2001; Imsland *et al.*, 2008). Result from the present study shows a significant increase in PCV of fish administered with *A. viridis* at 250 mg/kg, *A. viridis* 500 mg/kg and *S. mombin* 250 mg/kg compared with the control. The highest values of PCV, RBC, HB and WBC were recorded at the inclusion level of 250 mg/kg *A. viridis* among other treatments. This gives an indication that the plant extract contain some phytochemicals that can stimulate the formation or secretion of erythropoietin in the stem cells of the animals in the bone marrow to produce red blood cells (Ohlsson and Aher, 2012). The results from the present study has also shown a gradual and significant decrease of haematological parameters at higher dose of 500mg/kg of *A.*

*viridis* compared to the 250mg/kg. This phenomenon may be due to increasing toxicity of phytate, an antinutritive component of *A. viridis* that tend to overshadow the positive influence of the extract at higher concentration. Other antinutritive components present in *A. viridis* include tannins, oxalate and saponnin (Jonathan, 2013). The same trend was noticed for *S. mombin* where the lower dose of 250mg/kg had significantly higher haematological parameters than the 500mg/kg treated animals. Results of the present findings reported by previous studies which reported a decrease in haematocrit and haemoglobin with increase level of ingredients (Blom *et al.*, 2001; Dabrowski *et al.*, 2001; Rinchar *et al.*, 2003). Agus *et al.* (2013) also reported a reduction in growth performance and feed utilization from fish fed experimental diets with higher dietary katuk, a leaf vegetable grown in some tropic countries.

In this study there was significant increase in the thrombocyte value of *C. gariepinus* treated with *A. viridis* at 250 mg/kg. This is a significant observation because in fish, there is an important correlation between the number of blood thrombocytes and the clotting time (Tavares-Dias and Oliveira, 2009). This is because fish thrombocytes are sources of indispensable phospholipides that contribute to the activation of the coagulation factors, which activate the conversion of prothrombin to thrombin, which activates the fibrinogen that stimulates thrombocyte aggregation (Ranzani-Paiva *et al.*, 2000). Therefore, deviations in the number of thrombocytes and in the coagulation factors influence this process (Ranzani-Paiva *et al.*, 2000).

Previous study have shown that thrombocytes are involved in hemostasis and

organism defense, and are produced mainly by the spleen and kidney (Tavares-Dias and Oliveira, 2009). The significant increase in the groups administered with extracts as compared with control may indicate enhanced clotting time (thrombocytosis) in the event of any vascular injury to the exposed fish. This may be due to the extracts causing enhanced thrombocytopoiesis through increased rate of conversion of arachidonic acid to thromboxane B (Craig *et al.*, 2002).

In fish, liver is considered to be a major fat and glycogen deposition site (McClelland *et al.*, 1995; Péres and Oliva-Teles, 1999). Results of the present study have shown that liver was not affected by the dietary inclusion of extracts, since there was no significant difference in the hepatosomatic index. This is in agreement with a study where garlic, *Allium sativum* used as an immuostimulant and growth promoter in *Oreochromis niloticus* did not affect the HIS (Shalaby *et al.*, 2006). The higher viscerosomatic index obtained from *C. gariepinus* treated with *A. viridis* in the present study could be due to deposition of fat in the visceral. Increased visceral fat has been reported in *Tilapia mossambica* (Nair and Gopakumar, 1981), Mahseer fish (Bazaz and Keshavanath, 1993) and *Labeo rohita* administered with Livol a herbal product (Jayaprakas and Euphrasia, 1997).

Immunostimulants can increase non-specific immunity by either increasing the number of phagocytes or activating phagocytosis (Shoemaker *et al.*, 1997). White blood cell (WBC) and lymphocytes are the defensive cells of the body. According to Douglas and Jane (2010), higher the amount of White blood cell (WBC) and lymphocytes in the animal's body has an important implication in immune response and the ability to fight infections. Thus the higher the value of

WBC and lymphocytes, the better the ability of animals to fight infection. The significant increase in the total WBC of *C. gariepinus* treated with *A. viridis* 250 mg/kg compared to the other groups indicates stronger immune system toward invasion by foreign organism and prevention of infection. This may be due to the fact that *A. viridis* extract enhanced the development of liver and as a consequence increase in blood formation. The liver is the main blood forming organ in fish in addition to the spleen and fore-kidney. Iranloye (2002) also reported increases in total white blood cell count, neutrophils, lymphocytes and monocytes following 30-day of feeding garlic in rats.

Several herbs have been tested for their growth-promoting activities in aquatic animals (Jayaprakas and Eupharsia 1996; Citarasu *et al.*, 2002; Sivaram *et al.*, 2004). Platel *et al.* (2002) found that medicinal herbs are desirable for stimulating digestion, and had the highest stimulatory influence particularly on bile secretion and pancreatic enzymes activity. In another way, olfactory feed ingredients enhance growth through their ability to act as feeding enhancers for fish to eat more feed than normal (Adams, 2005). With the shift away from synthetic drugs, the use of medicinal herbs as an alternative for antibiotic growth-promoters in fish is becoming acceptable (Adedeji *et al.*, 2008). This is justified by the significantly higher percentage weight gain obtained in this study from *C. gariepinus* administered with *S. mombin* 250 mg/kg. This may be due to presence of saponins which are surface active sterols or triterpene glycoside compounds found in a variety of plants (Njoku and Akumefula, 2007). In spite of being toxic to cold-blooded organisms including fish at particular concentrations, saponin-rich plants may have potential for exploitation in fish production systems (Makkar *et al.*, 2007).



The dietary ginseng herb containing saponin triterpenoid glycosides called ginsenosides (or panaxosides) as active chemical components greatly enhanced the growth, diet utilization efficiency and haematological indices in Nile tilapia, *Oreochromis niloticus* fingerlings (Ashraf and Goda 2008).

The dose dependent decrease in the percentage weight gain for the two plants may be due to increasing levels of saponins and other antinutritive factors at higher doses of inclusion. Fish are said to have compensatory mechanism in their body system that can absorb the negative effect of antinutrients when the quantity is below certain threshold levels (Francis *et al.*, 2001). It is speculated that this may be due to excessive damage to the intestinal mucosa by the higher doses of extracts which may also be responsible for the 15% mortality recorded in *C. gariepinus* treated with *A. viridis* 500 mg/kg.

The significant decrease in the total microbial count among the treatment groups compared with control implied that these plants have a level of antimicrobial activities. The extract at *A. viridis* 500 mg/kg caused a drastic decrease in the total microbial count of the fish. From this present study, the higher doses (500mg/kg) of *A. viridis* and *S. mombin* exhibited the better antimicrobial potential. This is in agreement with the findings of Muhammad *et al.* (2012) that the leaf of *A. viridis* exhibited marked antimicrobial activities against food-borne and pathogenic bacteria *in vitro*. This activity might be due to the increasing levels of tannins, saponins and steroids and these secondary metabolites have been known for their effectiveness against many microbes (Mann *et al.*, 2008). Most of the effects observed with extract of *S. mombin* may be attributed to the constituent compounds of phenols, tannins,

anthraquinones and flavonoids presence in the plant (Ayoka *et al.*, 2005; 2006; Akubue *et al.*, 1983; Caraballo *et al.*, 2004; Corthout *et al.*, 1994). The presence of these active compounds has been reported for several activities like antibacterial (Ajao and Shonukan, 1985; Corthout *et al.*, 1994), antioxidant (Castner *et al.*, 1998), and anti-microbial (Verpoorte and Dihal, 1987; Corthout *et al.*, 1994; Abo *et al.*, 1999).

### **Conclusion and recommendation**

The extracts of *A. viridis* and *S. mombin* at 250 mg/kg may reduce stress, stimulate the immune system, increase protein synthesis and thus may confer a growth increase in fish particularly *C. gariepinus*. It is recommended from this study that a further study is required to isolate, characterize establish the effect of the principal active compounds from these extracts.

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