THE EFFECTS OF *VERNONIA AMAGDALINA* ON GROWTH AND OXIDATIVE STRESS PARAMETERS OF *CLARIAS GARIEPINUS* (BURCHELL 1822) EXPOSED TO DICLOFENAC

EZEOMEKE, Somadina Immaculata, NWAMBA, Helen Ogochukwu, CHIAHA, Emiliana Ijeoma and ANUKWU, John Uchenna
Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.

Corresponding Author: Ezeomeke, S. I. Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria. **Email:** somadina.ezeomeke@esut.edu.ng **Phone:** +234 816 859 2161

Received *January 26, 2024:* ***Revised*** *August 19, 2024;* ***Accepted*** *August 22, 2024*

ABSTRACT

Medicinal plants and plant extracts have been employed by rural fish farmers in fish management. This present study was designed to investigate the ameliorative effect of a medicinal plant, bitter leaf (Vernonia amagdalina) extract on African catfish (Clarias gariepinus) fingerlings contaminated with diclofenac using biometric and oxidative stress indices. The fingerlings were exposed to different concentrations of both bitter leaf extract (BLE) (2 and 6% body weight) and diclofenac (DCF) (0.3 and 0.4 mg/L) after a series of range-finding tests. The length and weight of the experimental fishes were taken weekly from different concentrations and were used to calculate the growth parameter. There were no significant differences ($p<0.05$) among the treatments in mean weight gain, specific growth rate, feed conversion ratio and condition factor when compared to their various control groups. Liver tissues of the experimental fishes were collected on the 14th and 28th day, from different concentrations and were assay for oxidative stress. The antioxidants: CAT (30.22 ± 0.148 to 66.50 ± 0.707) and MDA (22.61 ± 0.233 to 66.50 ± 3.536) increased significantly when compared to the control. The results indicated that the given concentration of diclofenac and V. amygdalina cause significant alteration in the oxidative stress of C. gariepinus and may have no adverse effect on the growth of C. gariepinus. However, this study did not show the detoxifying potential of V. amygdalina against the adverse effect of diclofenac in C. gariepinus.

Keywords: Aquatic ecosystem, Antioxidants, Catfish, Bitter leaf extract, Diclofenac, Growth, Weight

INTRODUCTION

Aquaculture is a rapidly growing agricultural sector, which produces animal protein, with an average annual increase of 6.2% per year in the period between 2000 – 2012 (FAO, 2020). In developing countries, aquaculture contributes greatly to economic growth by providing a source of income, food security, and livelihood (FAO, 2016). The occurrences of pharmaceuticals in aquatic environments have been arising as a problem and have become one of the major subjects of interest. The extensive use of pharmaceutical products in both human and veterinary medicine has led to contamination of aquatic ecosystems. According to Lonappan *et al*. (2016), pharmaceuticals are compounds, which include materials extensively used in medicine, agriculture, and biotechnology, such as drugs, antibiotics, and hormones. The intensive use of these chemicals has been reported, to have numerous disadvantages to both the environment and health such as resistance to pathogens, and the resurgence of pathogens (Reverter *et al.,*

2014; Caipang and Lazado, 2015), bioaccumulation and biomagnifications, which ultimately affect human health (Panwar *et al*., 2020). They reduce the quality of water and make it unfit for consumption. Through bioaccumulation, they accumulate in the muscles of fishes, other aquatic animals, aquatic plants, and sediment soil. They gradually enter the food chain through biomagnifications, consequently posing health risks to humans and other animals in the food chain. Bioaccumulation and biomagnifications of these chemical residues have reportedly led to acute and chronic diseases in animals and humans (Gill and Garg, 2014).

However, one of the common pharmaceutical drugs that contaminate the aquatic environment is diclofenac. Diclofenac is a therapeutic anti-inflammatory and analgesic drug in the world and one of the most common pharmaceutical drugs currently detected in the environment (UNESCO and HELCOM, 2017). It is one of the most used and widely sold anti-inflammatories and analgesics in the world and has been utilized for an extended period. It has been widely detected in aquatic environments and at concentrations that can be indicative of detrimental environmental effects (Fick *et al*., 2009). The presence of contaminants in aquatic environments, even at low concentrations, causes negative impacts on living organisms (Grenni *et al*., 2018; Kumar *et al*., 2019). Changes in these populations can affect the maintenance of biological processes and change the structure of the entire ecosystem (Grenni *et al*., 2018). There is a need to maintain control over the disposal of waste in water bodies (Yadav *et al*., 2019). Aquatic contamination has affected the quality of aquatic food produced and has caused great economic loss in fish. Thus, a need for measures to control the effect of contamination on fish using an environmentally friendly and biodegradable measure.

Recently, there has been an increasing interest in the use of medicinal plants and plant extract in aquaculture, though it has been in use by rural fish farmers. Numerous scientific studies have reported the positive impact of medicinal plants in aquaculture (Reverter *et al*., 2017). Nowadays, Medicinal plants are used in aquaculture to control diseases and improve fish production in Nigeria. Several researches on medicinal plants have shown various effects such as anti-stress, growth promotion, appetite stimulation, immune-stimulation, aphrodisiac, and anti-pathogen properties in fish and shrimp aquaculture due to the bioactive compounds they contain such as alkaloids, terpenoids, tannins, saponins and flavonoids (Reverter *et al*., 2017). Such plants are used to effectively boost growth, feed utilization, immunity, and disease resistance in fish species (Takaoka *et al*., 2011). These bioactive compounds account for their pharmacological effects (antioxidant, anti-diabetes, anti-inflammatory, anticancer, anti-malaria and others) which make them suitable for treating and preventing diseases in aquaculture.

Vernonia amygdalina is a medicinal plant known for its nutritional and medicinal value. It is one of the most popular medicinal plants in Africa and Asia. It is highly appreciated and consumed in various dishes; and used in soup preparation as a vegetable (Ogbono and Onugbo soups) in Nigeria and Cameron. According to research, *Vernonia amygdalina* contains different bioactive compounds, which accounts for its various pharmacological and biochemical properties. This makes it suitable for control of disease in aquaculture.

Clarias gariepinus, commonly known as African catfish, is a fish species that is commonly farmed in Nigeria. It is a hardy fish that tolerates both well and poorly oxygenated-waters, which makes it ideal for culture in areas with a limited water supply (Ayoola, 2008; Wing-Keong, 2021). It is prominent in culture because of its hardiness and fast growth rate. This study aims to examine the effects of Bitter leaf extract (BLE) on the growth and oxidative stress parameter of *C. gariepinus* treated with diclofenac.

MATERIALS AND METHODS

Experimental Plant Material and Preparation of Leaf Extract: The experiment was carried out at the Applied Biology Special Laboratory, Enugu State University of Science and Technology (ESUT), Agbani, Enugu, Enugu State. Fresh bitter leaves were obtained from a local farm and were identified (Dalziel, 1937) and authenticated by a taxonomist in the Applied Biology and Biotechnology Department, Enugu State University of Science and Technology, Enugu. The voucher specimen

(ESUT Herbarium CAB 2/Number 53) was kept in the Departmental herbarium for referral purposes. The leaves were washed with distilled water and air-dried for two weeks, then ground into powder using a sterile pestle and mortar, and stored in an air-tight container for further use. The ethanolic bitter leaf extract (BLE) was prepared according to Abdel-Shafi *et al*. (2019) and Omar *et al*. (2020). Bitter leaf powder (100 g) was extracted with 70% v/v aqueous ethanol for two hours. One hundred grams of bitter leaf powder yielded 20 g ethanolic extract.

Experimental Chemical: Diclofenac Potassium Tablets USP 50 mg with the brand name Chloflam 50, manufactured by McCoy Pharma Private Limited -12, MIDC, Tarapur, District, Palghar, Maharashtra-401506 India with the Batch No: MP9574, Serial No: OMCPL5AA6040 and NAFDAC Reg No: 04-5388, was used for the study.

Experimental Fish: One hundred and fifty (150) eight weeks old healthy *Clarias gariepinus* fingerlings with a mean weight of 1.31 ± 0.137g were purchased from Sacen Fish Farm, Enugu, Nigeria, and transported in a well-aerated 50 litres capacity aquarium tank to the Applied Biology Special Laboratory, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria. The fishes were acclimatized to laboratory conditions for two weeks using well water and were fed 3% of their body weight in divided rations, twice daily (8:30 am and 5:00 pm) with Skretting Catfish Starter Feed (45% crude protein and 2000 kcal/kg metabolisable energy), Skretting Nigeria, Ibadan, Nigeria.

Experimental Diets and Design: The feed was mixed with either distilled water or BLE according to Dandi *et al*. (2022) with little modification. One kilogram of the commercial feed was weighed into each of the three different basins. One of the basins held the control (CT) feed, to which 100 mL of distilled water was added and mixed thoroughly; the feed was pelletized and dried under shade, and later stored.

The experimental diets were made indoors by adding 2% (20 ml; low dose) and 6% (60 ml; high dose) BLE to the commercial feed.

The experimental diets were similarly pelletized and dried under shade, and stored in plastic bags in a refrigerator for later use.

After acclimatization, fish were randomly divided into nine groups; A (control) (0 ml), B (6% BLE + 0.4 DCF), C (6% BLE), and D (0.4 DCF) Group A (control, 0 ml), B (2% BLE), C (6% BLE), D (2% BLE + 0.3 mg/l DCF), E (6% BLE + 0.3 mg/l DCF), F (2% BLE + 0.4 mg/l DCF), G (6% BLE + 0.4 mg/l DCF), H (0.3 mg/l DCF) and I (0.4 mg/l DCF), and replicated thrice (10 fishes/replicate). The treatments were; Group A (control, 0 ml), H (0.3 mg/l DCF) and I (0.4 mg/l DCF) were fed with a control diet (CT) without and with exposure to 0.3 mg/l and 0.4 mg/l of diclofenac, respectively. Group B (2% BLE), D (2% BLE + 0.3 mg/l DCF) and F (2% BLE + 0.4 mg/l DCF) were fed with 2% BLE-supplemented diets without and with exposure to 0.3 mg/l and 0.4 mg/l of diclofenac, respectively. Group C (6% BLE), E (6% BLE + 0.3 mg/l DCF) and G (6% BLE + 0.4 mg/l DCF) were fed with 6% BLE-supplemented diets without and with exposure to 0.3 mg/l and 0.4 mg/l of diclofenac, respectively. Test fish were monitored at regular time intervals during the experimental period (28 days) and liver samples were taken on the 14th and 28th days for oxidative stress analysis.

Determination of Biometric and Production Parameter: Length in centimetres and weight in grams of fish, and feed consumption were obtained at weekly intervals. From the fish weights, length, and feed consumption, the following indices were determined.

Mean weight gain (MWG): MWG (g) = Average weight in grams / Number of days (Lawal *et al.*, 2013).

Specific growth rate (SGR): SGR = $W_2 - W_1$ x 100 / t, where W_2 = initial weight of the fish, W_1 = final weight of fish, and t = time in days (Eyo, 2003).

Feed conversion ratio (FCR): FCR = Feed consumed (g) / Weight gained by fish (g) (Solomon *et al.,* 2013).

Condition factor (K): The condition factor of catfish in the various treatments was determined using Fulton's condition factor (K) (Williams, 2000). K = W/ L^3 x 100/1, where W = weight of fish (g) and L = length of fish (cm) (Amisah *et al.,* 2009). The K value was calculated at the beginning and at the end of the experiment.

Assay of Antioxidant Enzymes

Determination of catalase activity: Catalase activity was assayed following the method described by Oyedemi *et al.* (2010). The percentage inhibition was evaluated following a decrease in absorbance at 620 nm. The liver was homogenized in 0.01 M phosphate buffer pH 7.0 and centrifuged at 5000 rpm. The reaction mixture consisted of 0.4 ml of hydrogen peroxide 0.2 M, 1 ml of 0.01 M phosphate buffer pH 7.0 and 0.1 ml of liver homogenate 10%w/v. The reaction of the mixture was stopped by adding 2 ml of dichromate acetic acid reagent and 5% Potassium dichromate ($K_2Cr_2O_7$) prepared in glacial acid. The changes in the absorbance were measured at 620 nm and recorded. Percentage inhibition was calculated using the equation: % Catalase inhibition = (normal activity – inhibited activity)/(normal activity) × 100%, where Normal activity = hydrogen peroxide + phosphate buffer and Inhibited activity = hydrogen peroxide + phosphate buffer + liver homogenate.

Estimation of lipid peroxidation: Lipid peroxidation in the liver was estimated calorimetrically by thiobarbituric acid reactive substances (TBARS) using the modification method of Niehaus Jr and Samuelsson (1968). In brief, 0.1 ml of liver homogenate (10%w/v) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCL reagents (thiobarbituric acid 0.37, 15, trichloroacetic acid and 0.25 N HCL). All the tubes were placed in a boiling water bath for 30 minutes and cooled. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535 nm against reference blank. The concentration of MDA was calculated using the equation: C = A/ E × L, Where A is the absorbance of the sample, E is the extinction coefficient (1.56×10^5 M^{-1} CM^{-1}) and L is the length of the light path (1 cm).

Data Analysis: The data from the feeding trial were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 21. Where significant differences were observed, the means were further separated using the Duncan New Multiple test range at a 5% level of significance. Student's t-test was employed to separate the differences between oxidative stress parameters on days 14 and 28.

RESULTS

Effect of Bitter Leaf Extract on the Weight of *Clarias gariepinus* Exposed to Diclofenac: The effect of BLE on the weight of fishes exposed to DCF, is presented in Table 1. The result on day 0, indicated that the fishes in Group A had the highest weight (1.41 ± 0.54 g) followed by fishes in Group I (1.38 ± 0.02 g). Furthermore, the fishes in Group D had the lowest weight (1.14 ± 0.28 g), followed by fishes in Group B (1.20 ± 0.31 g).

On day 28, the fishes in group I had the highest final weight (4.22 ± 0.75 g) followed by the fishes in group H (3.99 ± 0.14 g). Furthermore, the fishes in Group G had the lowest final weight (3.33 ± 0.38 g) followed by the fishes in Group B (3.45 ± 0.74 g).

Effect of Bitter Leaf Extract on the Weight Gain of *Clarias gariepinus* Exposed to Diclofenac: The effect of BLE on the weight gain of fishes exposed to DCF indicated that on day 7, fishes in Groups A and D had the highest weight gain (0.08 ± 0.05 and 0.08 ± 0.04 g respectively) followed by fishes in Group F (0.06 ± 0.04 g) (Table 2). Furthermore, the fishes in Group B had the lowest weight gain (0.02 ± 0.02 g) followed by fishes in Group G (0.04 ± 0.01 g).

On day 28, the fishes in Groups I and H had the highest weight gain (0.05 ± 0.02 and 0.05± 0.01 g respectively) followed by fishes in Groups B and D (0.04 ± 0.03 and 0.04 ± 0.01 g respectively). Furthermore, the fishes in Group G had the lowest weight gain (0.01 ± 0.03 g), followed by fishes in Group C (0.02 ± 0.02 g).

Table 1: The effect of *Vernonia amagdalina* (bitter leaf extract) on the weight of *Clarias gariepinus* (African catfish) exposed to diclofenac

Group	Weight of catfish (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
A (Control)	1.41 ± 0.54^{a4}	1.98 ± 0.51^{b3}	2.63 ± 0.37^{c6}	3.02 ± 0.50^{cd3}	3.83 ± 0.39^{d2}
B (2% BLE)	1.20 ± 0.31^{a2}	1.37 ± 0.17^{a1}	1.87 ± 0.18^{ab1}	2.45 ± 0.21^{b1}	3.45 ± 0.74^{c1}
C (2% BLE + 0.3 mg/L DCF)	1.31 ± 0.14^{a3}	1.61 ± 0.07^{a2}	2.22 ± 0.24^{b1}	2.87 ± 0.34^{c2}	3.50 ± 0.59^{d1}
D (2% BLE + 0.4 mg/L DCF)	1.14 ± 0.28^{a1}	1.67 ± 0.03^{b3}	2.31 ± 0.34^{c3}	2.46 ± 0.30^{c1}	3.71 ± 0.34^{d2}
E (6% BLE)	1.27 ± 0.03^{a2}	1.64 ± 0.19^{a2}	2.43 ± 0.42^{b}	2.68 ± 0.29^{b2}	3.54 ± 0.43^{c1}
F (6% BLE + 0.3 mg/L DCF)	1.37 ± 0.01^{a4}	1.78 ± 0.34^{b2}	2.36 ± 0.21^{bc3}	2.68 ± 0.48^{c2}	3.50 ± 0.60^{d1}
G (6% BLE + 0.4 mg/L DCF)	1.31 ± 0.01^{a3}	1.61 ± 0.04^{a2}	2.47 ± 0.06^{b4}	3.10 ± 0.68^{bc3}	3.33 ± 0.38^{c1}
H (0.3 mg/L DCF)	1.35 ± 0.02^{a3}	1.73 ± 0.10^{b3}	2.47 ± 0.32^{c4}	2.79 ± 0.25^{c2}	3.99 ± 0.14^{d3}
I (0.4 mg/L DCF)	1.38 ± 0.02^{a4}	1.69 ± 0.16^{a3}	2.50 ± 0.40^{b5}	2.89 ± 0.37^{b2}	4.22 ± 0.75^{c3}

^{abcd} = Means with different letter superscripts within each row differ significantly ($p \leq 0.05$), $^{1-6}$ = Means with varying superscripts of number within each column differ significantly ($p \leq 0.05$), Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac

Table 2: The effect of *Vernonia amagdalina* (bitter leaf extract) on the weight gain of *Clarias gariepinus* (African catfish) exposed to diclofenac

Group	Weight gain of catfish (g)			
	Day 7	Day 14	Day 21	Day 28
A (Control)	0.08 ± 0.05^{b5}	0.05 ± 0.01^{ab2}	0.02 ± 0.04^{a1}	0.03 ± 0.02^{a3}
B (2% BLE)	0.02 ± 0.02^{a1}	0.04 ± 0.02^{b1}	0.03 ± 0.01^{ab2}	0.04 ± 0.03^{b4}
C (2% BLE + 0.3 mg/L DCF)	0.05 ± 0.01^{b3}	0.05 ± 0.01^{b2}	$0.04 \pm 0.01a^{ab3}$	0.02 ± 0.02^{a2}
D (2% BLE + 0.4 mg/L DCF)	0.08 ± 0.04^{c5}	0.05 ± 0.02^{b2}	0.02 ± 0.02^{a1}	0.04 ± 0.01^{ab4}
E (6% BLE)	0.05 ± 0.02^{c3}	0.06 ± 0.03^{c3}	0.01 ± 0.03^{a1}	0.03 ± 0.03^{b3}
F (6% BLE +0.3 mg/L DCF)	0.06 ± 0.04^{c4}	0.04 ± 0.03^{b1}	0.02 ± 0.02^{a1}	0.03 ± 0.02^{b3}
G (6% BLE + 0.4 mg/L DCF)	0.04 ± 0.01^{b2}	0.06 ± 0.01^{c3}	0.03 ± 0.03^{b2}	0.01 ± 0.03^{a1}
H (0.3 mg/L DCF)	0.05 ± 0.02^{b3}	0.05 ± 0.02^{b2}	0.02 ± 0.02^{a1}	0.05 ± 0.01^{b5}
I (0.4 mg/L DCF)	0.05 ± 0.02^{b3}	0.06 ± 0.04^{b3}	0.02 ± 0.01^{a1}	0.05 ± 0.02^{b5}

^{abcd} = Means with different letter superscripts within each row differ significantly ($p \leq 0.05$), $^{1-5}$ = Means with varying superscripts of number within each column differ significantly ($p \leq 0.05$), Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac

Effect of Bitter Leaf Extract on the Specific Growth Rate of *Clarias gariepinus* Exposed to Diclofenac: The impact of BLE on the SGR of fishes exposed to DCF indicated that on day 7, fishes in Group A had the highest SGR (7.90 ± 4.51) followed by fishes in Group D (7.67 ± 3.88) (Table 3). Furthermore, the fishes in Group B had the lowest SGR (2.33 ± 2.09) followed by fishes in Group G (4.24 ± 1.16).

On day 28, the fishes in Group I had the highest SGR (4.77 ± 1.52) followed by fishes in Group D (4.45 ± 1.07). Furthermore, the fishes in Group G had the lowest SGR (0.81 ± 3.33), followed by fishes in Group C (2.25 ± 1.71).

The Effect of Bitter Leaf Extract on the Feed Conversion Ratio (FCR) of *Clarias gariepinus* Exposed to Diclofenac: The effect of BLE on the FCR of fishes exposed to DCF showed that on day 7, the fishes in Group B had the highest FCR (5.57 ± 6.10) followed by fishes in Group H (1.36 ± 0.15) (Table 4). Furthermore, the fishes in Group D had the lowest value (0.91 ± 0.38) followed by fishes in Group A (1.02 ± 0.89).

On day 28, the fish in Group F had the highest FCR (4.12 ± 6.31), followed by the fishes in Group E (2.32 ± 3.09) (Table 4). Furthermore, the fishes in Group G had the lowest FCR (0.24 ± 1.08) followed by fishes in Group I (0.36 ± 0.12).

The Effect of Bitter Leaf Extract on the Condition Factor (K) of *Clarias gariepinus* Exposed to Diclofenac: The effect of BLE on the K of fishes exposed to DCF showed that on day 0, fishes in Group B had the highest value of the K (1.24 ± 0.44)

Table 3: The effect of *Vernonia amagdalina* (bitter leaf extract) on the specific growth rate (SGR) of *Clarias gariepinus* (African catfish) exposed to diclofenac

Group	Specific growth rate of catfish			
	Day 7	Day 14	Day 21	Day 28
A (Control)	7.90 ± 4.51^{d4}	4.64 ± 1.15^{c2}	1.86 ± 3.91^{a2}	2.92 ± 2.37^{b2}
B (2% BLE)	2.33 ± 2.09^{a1}	3.60 ± 1.69^{b1}	2.76 ± 0.33^{ab3}	3.57 ± 2.85^{b3}
C (2% BLE + 0.3 mg/L DCF)	4.33 ± 1.07^{b2}	4.38 ± 1.22^{b2}	3.06 ± 0.48^{ab4}	2.25 ± 1.71^{a2}
D (2% BLE + 0.4 mg/L DCF)	7.67 ± 3.88^{c4}	4.57 ± 2.32^{b2}	0.71 ± 0.33^{a1}	4.45 ± 1.07^{b4}
E (6% BLE)	5.29 ± 2.36^{c3}	5.64 ± 3.42^{c3}	1.17 ± 3.08^{a2}	3.07 ± 2.46^{b3}
F (6% BLE +0.3 mg/L DCF)	5.95 ± 4.12^{d3}	4.12 ± 3.22^{c2}	1.54 ± 2.12^{a2}	2.90 ± 2.38^{b2}
G (6% BLE + 0.4 mg/L DCF)	4.24 ± 1.16^{c2}	6.14 ± 0.12^{d4}	3.02 ± 3.13^{b4}	0.81 ± 3.33^{a1}
H (0.3 mg/L DCF)	5.43 ± 1.73^{c3}	5.29 ± 1.58^{c3}	1.52 ± 1.98^{a2}	4.29 ± 1.24^{b4}
I (0.4 mg/L DCF)	4.52 ± 1.94^{b2}	5.76 ± 3.96^{c3}	1.84 ± 0.74^{a2}	4.77 ± 1.52^{b4}

^{abcd} = Means with different letter superscripts within each row differ significantly (p≤0.05), $^{1-4}$ = Means with different number superscripts within each column differ significantly (p≤0.05), Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac

Table 4: The effect of *Vernonia amagdalina* (bitter leaf extract) on the feed conversion ratio (FCR) of *Clarias gariepinus* (African catfish) exposed to diclofenac

Group	Feed conversion ratio of catfish			
	Day 7	Day 14	Day 21	Day 28
A (Control)	1.02 ± 0.89^{c1}	0.57 ± 0.13^{b1}	0.16 ± 0.92^{a1}	1.42 ± 1.79^{d5}
B (2% BLE)	5.57 ± 6.10^{b3}	0.98 ± 0.70^{a3}	0.87 ± 0.28^{a34}	0.78 ± 0.36^{a4}
C (2% BLE + 0.3 mg/L DCF)	1.31 ± 0.46^{b2}	0.50 ± 0.04^{a1}	0.57 ± 0.09^{a2}	1.29 ± 1.47^{b5}
D (2% BLE + 0.4 mg/L DCF)	0.91 ± 0.38^{b1}	0.96 ± 0.53^{b3}	3.30 ± 0.88^{c}	0.48 ± 0.17^{a3}
E (6% BLE)	1.08 ± 0.50^{c1}	0.84 ± 0.70^{b23}	0.52 ± 1.80^{a2}	2.32 ± 3.09^{d6}
F (6% BLE +0.3 mg/L DCF)	1.12 ± 0.56^{b1}	2.60 ± 3.52^{c4}	0.70 ± 3.23^{a3}	4.12 ± 6.31^{d7}
G (6% BLE + 0.4 mg/L DCF)	1.16 ± 0.25^{c1}	0.54 ± 0.01^{b1}	1.32 ± 0.95^{c5}	0.24 ± 1.08^{a1}
H (0.3 mg/L DCF)	1.36 ± 0.15^{c2}	0.73 ± 0.17^{b2}	1.44 ± 4.28^{c5}	0.42 ± 0.06^{a3}
I (0.4 mg/L DCF)	1.28 ± 0.59^{c2}	0.91 ± 0.81^{b3}	0.93 ± 0.42^{b4}	0.36 ± 0.12^{a2}

^{abcd} = Means with different letter superscripts within each row differ significantly (p≤0.05), $^{1-7}$ = Means with varying superscripts of number within each column differ significantly (p≤0.05), Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac

followed by fishes in Group E (1.22 ± 0.57) (Table 5). The fishes in Group A had the lowest value (0.94 ± 0.10) followed by fishes in Group F (0.95 ± 0.18).

On day 28, the fishes in Group C had the highest value of the k (1.36 ± 0.45), followed by fishes in Group D (1.10 ± 0.24) (Table 5). Furthermore, the fishes in Group B had the lowest value (0.71 ± 0.22) followed by fishes in Group I (0.78 ± 0.18).

Effect of Bitter Leaf Extract on the Oxidative Stress Parameter of *Clarias gariepinus* Exposed to Diclofenac: The effect of BLE on the oxidative stress parameter of *C. gariepinus* indicated that on day 14, Malondialdehyde (MDA) had the lowest value in control (12.77 ± 0.02) and the highest value in 6% BLE + 0.4 DCF (26.50 ± 0.38). Furthermore, on day 28, MDA had its lowest value in control (20.84 ± 0.23), and its highest value in 6% BLE + 0.4 DCF (66.50 ± 3.54).

Catalase, on day 14, had the lowest value in control (18.12 ± 0.01) and the highest value in 6% BLE + 0.4 DCF (45.67 ± 0.07). Furthermore, on day 28, catalase had its lowest value in the control (20.85 ± 1.34), and its highest value in 6% BLE + 0.4 DCF (66.50 ± 0.71).

DISCUSSION

Growth of *Clarias gariepinus*: Fish growth depends on feed intake and a host of other intrinsic and extrinsic factors (Eyo, 2003).

Table 5: The effect of *Vernonia amagdalina* (bitter leaf extract) on the condition factor of *Clarias gariepinus* (African catfish) exposed to diclofenac

Group	Condition factor of catfish				
	Day 0	Day 7	Day 14	Day 21	Day 28
A (Control)	0.94 ± 0.10^{ab1}	0.79 ± 0.16^{a1}	0.97 ± 0.35^{ab2}	1.12 ± 0.29^{b2}	0.98 ± 0.45^{ab2}
B (2% BLE)	1.24 ± 0.44^{b2}	0.99 ± 0.25^{ab2}	0.90 ± 0.29^{ab2}	1.24 ± 0.34^{b3}	0.71 ± 0.22^{a1}
C (2% BLE + 0.3 mg/L DCF)	1.01 ± 0.34^{b2}	0.77 ± 0.06^{a1}	1.08 ± 0.23^{b3}	1.04 ± 0.05^{b2}	1.36 ± 0.45^{b3}
D (2% BLE + 0.4 mg/L DCF)	1.19 ± 0.60^{ab2}	1.12 ± 0.17^{a3}	1.27 ± 0.12^{b3}	1.26 ± 0.49^{b3}	1.10 ± 0.24^{a2}
E (6% BLE)	1.22 ± 0.57^{b2}	1.17 ± 0.28^{b3}	0.97 ± 0.28^{ab2}	0.83 ± 0.14^{a1}	0.85 ± 0.05^{a1}
F (6% BLE + 0.3 mg/L DCF)	0.95 ± 0.18^{b1}	0.95 ± 0.19^{b2}	0.92 ± 0.21^{ab2}	0.87 ± 0.11^{a1}	0.93 ± 0.40^{ab2}
G (6% BLE + 0.4 mg/L DCF)	1.03 ± 0.31^{b2}	0.92 ± 0.26^{ab2}	0.76 ± 0.13^{a1}	1.05 ± 0.13^{b2}	1.03 ± 0.14^{b2}
H (0.3 mg/L DCF)	1.12 ± 0.17^{b2}	1.20 ± 0.21^{b3}	0.80 ± 0.22^{a1}	1.20 ± 0.27^{b3}	0.92 ± 0.25^{ab2}
I (0.4 mg/L DCF)	0.99 ± 0.11^{b1}	0.77 ± 0.11^{a1}	0.93 ± 0.10^{b2}	0.91 ± 0.23^{b}	0.78 ± 0.18^{a1}

^{abcd} = Means with different letter superscripts within each row differ significantly ($p \leq 0.05$), $^{1-3}$ = Means with varying superscripts of number within each column differ significantly ($p \leq 0.05$), Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac

Table 6: The effect of *Vernonia amagdalina* (bitter leaf extract) on the oxidative stress parameter of *Clarias gariepinus* (African catfish) exposed to diclofenac

Parameters	Exposure time	Oxidative stress (mg/L)			
		Control	6% BLE + 0.4 DCF	6% BLE	0.4 DCF
Lipid Peroxidation	14 days	12.77 ± 0.02^{a}	26.50 ± 0.38^{d}	23.39 ± 0.24^{c}	22.61 ± 0.23^{b}
	28 days	$20.84 \pm 0.23^{a*}$	$66.50 \pm 3.54^{c*}$	$53.50 \pm 2.12^{b*}$	$56.00 \pm 1.41^{b*}$
Catalase	14 days	18.12 ± 0.01^{a}	45.67 ± 0.07^{d}	37.42 ± 0.03^{c}	30.22 ± 0.15^{b}
	28 days	$20.85 \pm 1.34^{a*}$	$66.50 \pm 0.71^{c*}$	$64.00 \pm 1.41^{bc*}$	$61.50 \pm 2.12^{b*}$

*Means with different letter superscripts within each row differ significantly ($p \leq 0.05$). Data are presented as mean ± SE, BLE – Bitter leaf extract, DCF = Diclofenac, * = significant mean ($p < 0.05$) of the oxidative parameters between days 14 and 28 using student's t-test pairwise comparison*

There were no significant differences ($p < 0.05$) in the growth parameters among the treatments when compared to the control. This was in line with the report of Udoh *et al.* (2017), which observed no significant difference in feed conversion ratio, initial weight, and specific growth rate of *C. gariepinus* fed with *V. amygdalina* at varying proportions. Okukpe *et al.* (2018) reported a significant decrease in feed intake and growth rate in *C. gariepinus* fed with supplementation of *V. amygdalina* leaf meal. This finding was in contrast to Dandi *et al*. (2022), who reported a significant increase in the growth of Nile Tilapia fed with *V. amygdalina* aqueous extract. Reports have shown improvements in growth and feed utilization resulting from improved metabolism by incorporating lower doses of medicinal plants (Güroy *et al*., 2012; Celikbilek *et al*., 2013; Dadras *et al*., 2019; Dandi *et al*., 2022). An increase in *V. amygdalina* concentration decreased feed palatability due to the bitterness of BLE (Ezenwanne and Ucheya 2012; Dandi *et al*., 2022). Praskova *et al*. (2014) reported significant decreases in the growth of Zebra fish treated with diclofenac. Van den Brandhof and Montforts (2010) found growth retardation, delayed hatching, and yolk sac and tail deformation of Zebra fish in concentrations of diclofenac above 1.5 mg/L.

Oxidative Stress of *Clarias gariepinus*: Oxidative stress is a situation when an organism's internal resistance to anti-oxidative stress enzymes has yielded to the onslaught of reactive oxygen species (Folarin *et al*., 2018). Lipid peroxidation in the fishes treated with BLE and DCF was significantly higher than the control, with 6% BLE + 0.4 DCF having the highest value on days 14 and 28.

The increase in lipid peroxidation may be attributed to the ability of *V. amygdalina* and DCF to enhance the production of reactive oxygen species (ROS). The level of lipid peroxidation in the liver tissue reflects increased oxidative stress

and lipoperoxidation. Cellular oxidative stress results when the balance between pro-oxidants and antioxidants is disrupted leading to excessive generation of reactive oxygen (Dabas *et al*., 2012). The interaction of ROS with biological molecules may cause an increase in lipid peroxidation, DNA damage, and protein oxidation resulting in the disturbance of the physiological processes (Tejada *et al*., 2007).

The increase in lipid peroxidation was in agreement with Piner *et al*. (2007) and Eze *et al*. (2021), who reported an increase in lipid peroxidation in *Oreochromis niloticus* exposed to fenthion and diclofenac. The increase in lipid peroxidation may be due to the stress associated with exposure to the DCF and *V. amygdalina* earlier reported in catfish administered albendazole (Nwani *et al*., 2016). Some related pharmaceuticals, notably benznidazole and mebendazole have been reported to stimulate the production of ROS and to cause oxidative damage and lipid peroxidation in animals (Führ *et al*., 2012; Nwani *et al*., 2016). Antioxidant enzymes play significant roles in preventing cellular damage in animals (Nwani *et al*., 2016).

The cells have efficient mechanisms for combating the effects of oxidative stress and repairing damaged macromolecules produced during exposure to pollutants. Enzymatic (catalase) antioxidants provide an adequate defence and help to scavenge ROS. The catalase activities in the fishes treated with BLE and DCF were significantly higher than the control, with 6% BLE + 0.4 DCF having the highest value on days 14 and 28. The increase in catalase activity may be in response to the damaging effects of H_2O_2 which results from the degradation of anion superoxide by the enzyme superoxide dismutase

This was in contrast to the report by Folarin *et al*. (2018) and Eze *et al*. (2021), where the catalase activities in the fish exposed to diclofenac were significantly lower than the control. Catalase activity has increased in *C. gariepinus* exposed to butachlor (Farombi *et al*., 2007) and *Prochilodus lineatus* exposed to glyphosate-based herbicides (Caramello *et al*., 2017). The limited capacity of the antioxidants in fish to neutralize the effects could impair liver functions (Dabas *et al*., 2012). The higher values of these parameters in the liver may also be attributed to the high metabolic reaction and free radical generation in the liver that requires the presence of antioxidants for possible protection against the oxidative stress induced by ROS (Pereira *et al*., 2013)

Conclusion: This present study has shown that the given concentration of diclofenac and *V. amygdalina* can cause significant alteration in the oxidative stress of *C. gariepinus*. Thus, it can be deduced that diclofenac at various concentrations and duration of study can cause adverse effects on vital animal tissues, resulting in oxidative disorder. The result showed that the given concentration of diclofenac may have no adverse effect on the growth of *C. gariepinus*. Thus, caution should be exercised in the clinical use of diclofenac for therapeutic purposes, which should be limited to the lowest dose and treatment duration required to achieve the best therapeutic effect to avoid having adverse effects on non-target organisms. *V. amygdalina,* though a medicinal plant that is rich in bioactive compounds, can cause oxidative stress in *C. gariepinus* at high concentrations. The result from this study indicated that dietary supplementation with *V. amygdalina* at the given concentration had no significant effect on growth. However, *V. amygdalina* should be administered at a lower concentration than *C. gariepinus*. Hence, this current study has not shown the detoxifying potential of *V. amygdalina* against the adverse effect of diclofenac in *C. gariepinus*. However, more research is required to determine the safe level of *V. amygdalina* on *C. gariepinus* and the extraction type that best improves *C. gariepinus* health more effectively. It may be necessary to investigate the possible effect of *V. amygdalina* in increasing levels of fishes treated with diclofenac on amino acid profile and other parameters, such as haematology, histopathology, biochemistry, metabolism, and digestion.

ACKNOWLEDGEMENTS

The authors thank the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, for the immense support and Mr. Job Uka of Fedrah

Research Institute for his technical support. We also thank Prof. Joseph Eyo for his assistance in the preparation of this manuscript.

REFERENCES

ABDEL-SHAFI, S., AL-MOHAMMADI, A. R., SITOHY, M., MOSA, B., ISMAIEL, A., ENAN, G. and OSMAN, A. (2019). Antimicrobial activity and chemical constitution of the crude, phenolic-rich extracts of *Hibiscus sabdariffa, Brassica oleracea,* and *Beta vulgaris*. *Molecules*, 24(23): 4280. https://doi.org/10.3390/molecules24234280

AMISAH, S., OTENG, M. A. and OFORI, J. K. (2009). Growth performance of the African catfish, *Clarias gariepinus*, fed varying inclusion levels of *Leucaena leucocephala* leaf meal. *Journal of Applied Sciences and Environmental Management*, 13(1): 21 – 26.

AYOOLA, S. O. (2008). Histopathological effect of glyphosate on juvenile African catfish (*Clarias gariepinus*). *Journal of Agricultural and Environmental Science*, 4(3): 362 – 367.

CARAMELLO, C. S., JORGE, M. J., JORGE, N. L. and JORGE, L. C. (2017). Evaluation of herbicide glyphosate effects in the fish *Prochilodus lineatus* using chromosome aberration test. *Revista Veterinaria*, 28(1): 65 – 68.

CELIKBILEK, M., DOGAN, S., OZBAKIR, O., ZARARSIZ, G., KÜCÜK, H., GÜRSOY, S., YURCI, A., GÜVEN, K. and YÜCESOY, M. (2013). Neutrophil–lymphocyte ratio as a predictor of disease severity in ulcerative colitis. *Journal of Clinical Laboratory Analysis*, 27(1): 72 – 76.

DABAS, A., NAGPURE, N. S., KUMAR, R., KUSHWAHA, B., KUMAR, P. and LAKRA, W. S. (2012). Assessment of tissue-specific effect of cadmium on antioxidant defense system and lipid peroxidation in freshwater murrel, *Channa punctatus*. *Fish Physiology and Biochemistry*, 38(2): 469 – 482.

DADRAS, H., HAYATBAKHSH, M. R. and GOLPOUR, A. (2019). Dietary administration of common sage (*Salvia officinalis*) and coneflower (*Echinacea angustifolia*) extracts affects growth, blood parameters and immune responses of Beluga, *Huso huso. Turkish Journal of Fisheries and Aquatic Sciences*, 20(5): 367 – 374.

DALZIEL, J. M. (1937). *The Useful Plants of West Tropical Africa*. Crown Agents for the Colonies, London.

DANDI, S. O., ABARIKE, E. D. and AMPOFO-YEBOAH, A. (2022). Bitter leaf *Vernonia amygdalina* extract enhances growth, hematology, heat stress response, and resistance to *Aeromonas hydrophila* in Nile tilapia. *North American Journal of Aquaculture*, 84(4): 432 – 441.

EYO, J. E. (2003). Acceptability, growth performance and cost analysis of diets enriched with lipids from varied plants and animal sources fed to fingerlings of *Clarias gariepinus* (Teleostei, Clariidae) Burchell, 1822. *Bio-Research*, 1(2): 87 – 100.

EZE, C. C., NWAMBA, H. O., OMEJE, F. U., ANUKWU, J. U., OKPE, M. N. and NWANI, C. D. (2021). Effects of diclofenac on the oxidative stress parameters of freshwater fish *Oreochromis niloticus. Journal of Applied Life Sciences International*, 24(10): 44 – 51.

EZENWANNE, E. B. and UCHEYA, R. E. (2012). A study of the serum concentrations of some hepatic enzymes in doses of aqueous leaf extract of *Vernonia amygdalina* in rabbits. *International Journal of Animal and Veterinary Advances*, 4(2): 80 – 83.

FAO (2016). *The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All*. Food and Agriculture Organization of the United Nations, Rome, Italy. https://www.fao.org/3/i5555e/i5555e.pdf

FAO (2020). *The State of World Fisheries and Aquaculture 2020: Sustainability in Action.* Food and Agriculture Organization of the United Nations, Rome, Italy. https://www.fao.org/3/ca9229en/ca9229en.pdf

FAROMBI, E. O., ADELOWO, O. A. and AJIMOKO, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African catfish (*Clarias gariepinus*) from Nigeria Ogun River. *International Journal*

of Environmental Research and Public Health, 4(2): 158 – 165.

FICK, J., SÖDERSTRÖM, H., LINDBERG, R. H., PHAN, C., TYSKLIND, M. and LARSSON, D. J. (2009). Contamination of surface, ground, and drinking water from pharmaceutical production. *Environmental Toxicology and Chemistry*, 28(12): 2522 – 2527.

FOLARIN, O., OTITOLOJU, A. A. and AMAEZE, N. H. J. (2018). Comparative ecotoxicological assessment of acetaminophen and diclofenac using freshwater African catfish *Clarias gariepinus* (Burchell 1822). *Journal of Applied Sciences and Environmental Management,* 22(9): 1523 – 1529.

FÜHR, F., PEREIRA JUNIOR, J., ROMANO, L. A. and ALMEIDA, F. D. M. (2012). Gill injury after treatment with mebendazole on mullets *Mugil liza. Bulletin of European Association of Fish Pathology,* 32: 151 – 158.

GILL, H. K. and GARG, H. (2014). Pesticide: environmental impacts and management strategies. In: LARRAMENDY, M. L. and SOLONESKI, S. (Eds.). *Pesticides-Toxic Aspects*. IntechOpen, London.

GRENNI, P., ANCONA, V. and CARACCIOLO, A. B. (2018). Ecological effects of antibiotics on natural ecosystems: A review. *Microchemical Journal*, 136: 25 – 39.

GÜROY, B., ŞAHIN, İ., MANTOĞLU, S. and KAYALI, S. (2012). *Spirulina* as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid *Pseudotropheus acei*. *Aquaculture International*, 20: 869 – 878.

KUMAR, M., JAISWAL, S., SODHI, K. K., SHREE, P., SINGH, D. K., AGRAWAL, P. K. and SHUKLA, P. (2019). Antibiotics bioremediation: Perspectives on its ecotoxicity and resistance. *Environment International*, 124: 448 – 461.

KUMAR, R., SANKHLA, M. S., KUMAR, R. and SONONE, S. S. (2021). Impact of pesticide toxicity in aquatic environment. *Biointerface Research in Applied Chemistry*, 11(3): 10131 – 10140.

LAWAL, M. O., ADEROLU, A. Z., AARODE, O. O. and YEKINNI, A. (2013). Growth and economic performance of *Clarias gariepinus* fed different sources of calcium and phosphorus diets. *Journal of Fisheries Sciences.com,* 7(2): 187 – 193.

LONAPPAN, L., BRAR, S. K., DAS, R. K, VERMA, M. and SURAMPALL, R. Y. (2016). Diclofenac and its transformation products: environmental occurrence and toxicity - a review. *Environment International*, 96: 127 – 138.

NIEHAUS JR, W. G. and SAMUELSSON, B. (1968). Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry*, 6(1): 126 – 130.

NWANI, C. D., ODO, G. E., NWADINIGWE, A. O., ONYEKA, C. C., ATTAMA, C. I., NGWU, G., OLUAH, S. N., UKONZE, J. A. and EZEIBE, B. C. A. (2016). Short-term effects of albendazole on the oxidative stress markers and haematological parameters in tissues of African catfish (*Clarias gariepinus*). *Journal of Aquatic Animal Health,* 28(4): 222 – 228.

OKUKPE, K. M., YUSUF, O. A., ADEYEMO, A. D., OMOTAYO, O. O., ADEYINA, A. O., ALLI, I. O., ADEYEMI-ALE, O. A., ADERIBIGBE, T. A., OPOWOYE, I. O., OGUNSOLA, F. O. and MORAKINYO, O. S. (2018). Effects of *Vernonia amygdalina* leaf powder on growth performance of *Clarias gariepinus. Nigerian Journal of Animal Science*, 20(3): 180 – 188.

OMAR, A. E., AL-KHALAIFAH, H. S., MOHAMED, W. A., GHARIB, H. S., OSMAN, A., AL-GABRI, N. A. and AMER, S. A. (2020). Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behaviour, intestinal histology, amino acid digestibility, antioxidant activity, and the immune status of broiler chickens. *Frontiers in Veterinary Science*, 7: 582612. https://doi.org/10.3389/fvets.2020.582612

OYEDEMI, S. O., BRADLEY, G. and AFOLAYAN, A. J. (2010). In-vitro and-vivo antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. *African Journal of Pharmacy and Pharmacology*, 4(2): 70 – 78.

PANWAR, S., THAPLIYAL, A. and THAPLIYAL, M. (2020). A review on role of medicinal plants-based remedies in fish pathology. *Ecology, Environment and Conservation*, 26: S83 – S89.

PEREIRA, L., FERNANDES, M. N. and MARTINEZ, C. B. (2013). Hematological and biochemical alterations in the fish *Prochilodus lineatus* caused by the herbicide clomazone. *Environmental Toxicology and Pharmacology*, 36(1): 1 – 8.

PINER, P., SEVGILER, Y. and ÜNER, N. (2007). In vivo effects of fenthion on oxidative processes by the modulation of glutathione metabolism in the brain of *Oreochromis niloticus*. *Environmental Toxicology: An International Journal*, 22(6): 605 – 612.

PRASKOVA, E., PLHALOVA, L., CHROMCOVA, L., STEPANOVA, S., BEDANOVA, I., BLAHOVA, J., HOSTOVSKY, M., SKORIC, M., MARŠÁLEK, P., VOSLAROVA, E. and SVOBODOVA, Z. (2014). Effects of subchronic exposure of diclofenac on growth, histopathological changes, and oxidative stress in zebrafish (*Danio rerio*). *The Scientific World Journal*, 2014: 645737. https://doi.org/10.1155/2014/645737

REVERTER, M., BONTEMPS, N., LECCHINI, D., BANAIGS, B. and SASAL, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433: 50 – 61.

REVERTER, M., TAPISSIER-BONTEMPS, N., SASAL, P. and SAULNIER, D. (2017). Use of medicinal plants in aquaculture. AUSTIN, B. and NEWAJ-FYZUL, A. (Eds.). *Diagnosis and control of Diseases of Fish and Shellfish*. Wiley, Chichester, United Kingdom.

SOLOMON, S. G., ATAGUBA, G. A. and LMBUR, I. (2013). Growth performance of juvenile *Clarias gariepinus* fed different dietary lipid sources. *International Journal of Research on Fisheries and Aquaculture,* 12: 50 – 54.

TAKAOKA, O., JI, S. C., ISHIMARU, K., LEE, S. W., JEONG, G. S., ITO, J., BISWAS, A. and TAKII, K. (2011). Effect of rotifer enrichment with herbal extracts on growth and resistance of red sea bream, *Pagrus major* (Temminck and Schlegel) larvae against *Vibrio anguillarum*. *Aquaculture Research*, 42(12): 1824 – 1829.

TEJADA, S., SUREDA, A., ROCA, C., GAMUNDÍ, A. and ESTEBAN, S. (2007). Antioxidant response and oxidative damage in brain cortex after high dose of pilocarpine. *Brain Research Bulletin*, 71(4): 372 – 375.

UDOH, J. P., EMAH, A. U., GEORGE, I. E. and PHILIP, A. E. (2017) Growth performance and haematological response of *Clarias gariepinus* broodstock fed diets enriched with bitter leaf meal. *AACL Bioflux*, 10(5):1281 – 1296.

UNESCO and HELCOM (2017). *Pharmaceuticals in the Aquatic Environment of the Baltic Sea Region – A Status Report*. UNESCO Emerging Pollutants in Water Series 1, UNESCO Publishing, Paris.

USYDUS, Z., SZLINDER-RICHERT, J., ADAMCZYK, M. and SZATKOWSKA, U. (2011). Marine and farmed fish in the Polish market: Comparison of the nutritional value. *Food Chemistry*, 126(1): 78 – 84.

VAN DEN BRANDHOF, E. J. and MONTFORTS, M. (2010). Fish embryo toxicity of carbamazepine, diclofenac and metoprolol. *Ecotoxicology and Environmental Safety*, 73(8): 1862 – 1866.

WILLIAMS, J. E (2000). The coefficient of condition of fish. Chapter 13. *In:* SCHNEIDER, J. C. (Ed.). *Manual of Fisheries Survey Method 2 Periodic Updates*. Fisheries Special Report 25, Michigan Department of Natural Resources, Ann Arbor, Michigan, USA.

WING-KEONG N. (2021). *Clarias gariepinus*. CABI Compendium. https://doi.org/10.1079/cabicompendium.88683

YADAV, H., SANKHLA, M. S. and KUMAR, R. (2019). Pesticides-induced carcinogenic and neurotoxic effect on human. *Forensic Research and Criminology International Journal*, 7(5): 243 – 245.