DOI: http://dx.10.15578/iaj.19.1.2024.25-34 *Indonesian Aquaculture Journal, 19 (1), 2024 ,25-34*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

THE EFFECT OF DIETARY SUPPLEMENTATION OF *Sargussum wightii* EXTRACT ON *Clarias gariepinus* PHYSIOLOGICAL RESPONSES

Ademola Zaid Aderolu[*)], Ismail O. Ishola[**)], Uzeme Precious Aluta[*)#], and Boluwatife O. Williams[*)]

[*)]Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
[**)]Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine of the University of Lagos, Idi-araba, Lagos, Nigeria

(Received: October 31, 2023; Final revision: December 18, 2023; Accepted: December 22, 2023)

ABSTRACT

Seaweeds are becoming vital feed materials due to their high concentration of nutrients and active secondary metabolites. In this context, the brown seaweed *Sargassum wightii* extract (SWE) was included in *Clarias gariepinus* diets to investigate its effects on growth rate, nutrient utilization, haemato-biochemical indices, antioxidant activities and immune responses. Four experimental diets with different levels (control- 0 %, 0.25 %, 0.5 %, and 1.0 %) of SWE were fed to four experimental groups of *C. gariepinus* juvenile (mean initial weight 14.50 ± 0.00 g) for 28 days. At the end of the feeding trial, there was no significant difference ($p>0.05$) in the growth performance of fish groups fed SWE and the control group. However, there were significant differences ($p<0.05$) in feed conversion ratio and protein efficiency ratio between the control and SWE groups. Erythrocytes, leukocytes, haemoglobin, lymphocytes, and high-density lipoprotein cholesterol in fish fed SWE supplemented diets were significantly higher ($p<0.05$) than control. Notably, the liver function enzyme such as alkaline phosphatase was significantly higher ($p<0.05$) in fish fed SWE diets when compared to the control group. There was higher significant variation ($p<0.05$) in hepatic antioxidant enzymes activities across all diets. It was also observed that TNF-á, IL-6 and IL-â increased significantly in the liver of *C. gariepinus* fed SWE supplemented diets compared to control. Therefore, these results suggest that inclusion of *S. wightii* extract in the fish diet could improve the immune status and reduced oxidative stress of *C. gariepinus*.

KEYWORDS: C. gariepinus; S. wightii; Phytochemicals; Antioxidative properties; Blood profile; Immunostimulatory effects

INTRODUCTION

In recent years, there has been a focus on environmentally friendly and sustainable techniques of aquaculture disease management procedures. Such sustainable technique includes the use of terrestrial plant and medicinal herbs as supplements and/or antibiotics rather than antibiotics and chemicals in aquaculture due to several factors including lower cost, readily available, lesser side effects on fish health, and the aquatic environments (Reverter *et al.*, 2014). Furthermore, marine flora, although being underexploited, also represents abundant sources of carotenoids, polysaccharides, fatty acids, acetogenins, amino acids, peptides, vitamins, and phenolic compounds (La Barre *et al.*, 2010; Moghadamtousi *et al.*, 2015). Specifically, marine macroalgae also known as seaweed consist of variety of bioactive substances that have persisted in them through evolutions and have enabled them to adapt to a variety of environmental challenges, for example, substances such as carotenoids present in seaweeds protect them from excessive light, tocopherols protect them from oxidative stress, phycocyanins and phycobiliproteins are used in defence against pathogens and glycerol protect from osmotic stress caused by changes in salinity (Kumar *et al.*, 2008). These secondary metabolites have been found to possess vast biologically activities (antiviral, anthelmintic, antifungal, and antibacterial) that could render them valuable for application as pharmaceuticals and nutraceuticals in the aquaculture industries (Shibata *et al.*, 2002; Giddings *et al.*, 2015).

The brown seaweeds, one of the three main taxonomic groups, are receiving a lot of interest since their areal production is substantially higher than that of green or red algae, they may contain a higher amount of phenolic chemicals, a complex type of polysaccharide, exceptionally high biological activity,

[#] Correspondence: Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
E-mail: ualuta@unilag.edu.ng

and a more powerful antioxidant capacity than the green and red seaweed. (Generaliæ *et al*., 2019). *Sargassum*, a genus of brown seaweed (Phaeophyta) in the family Sargassaceae, comprises of about 400 species. *Sargassum* species are floating seaweed distributed widely across the world's oceans and are important because of their dietary and medicinal values (Mattio & Payri, 2011). *Sargassum wightii*, found in shallow coastal waters in the littoral and sublittoral areas of the tropical region throughout the year, is an important species of the family (Devi *et al*., 2014; Deepak *et al*., 2018). *S. wightii* contains large amount of natural bioactive compounds such as flavonoids, polyphenols and alkaloids that could promote its antimicrobial, antioxidant and immunostimulatory activities and therefore justifies its applications as feed supplements to promote the well-being of cultured fish species (Deepika, 2017; Almeida *et al*., 2020; Afreen *et al*., 2023). Previous studies had reported that *Sargassum* spp. extracts possesses potent antibacterial activities against aquaculture important fish pathogens (Baleta *et al*., 2011; Bolaños *et al*., 2017).

Clarias gariepinus (Burchell, 1822) is a widely distributed cultured fish species which is easily available and highly valuable in sub-Saharan Africa for many reasons including easy propagation, rapid growth, and consumers' demand (Dauda *et al*., 2018). In addition, *C. gariepinus* is widely cultured due to its resilience and high adaptiveness. Nevertheless, disease outbreak in catfish farms that are caused by infectious organisms have been reported (Mbokane *et al*., 2022). Since, the use of antibiotics and chemicals have been restricted for use in fish farming, it is of utmost importance to identify eco-friendly and biodegradable feed supplements that could sustainably improve the health status of *C. gariepinus*. Hence, the aim of this study was to investigate the effect of ethanolic extract of *Sargassum wightii* on the growth performance, nutrient utilization and health status of *Clarias gariepinus.*

MATERIALS and METHODS

Sample collection

Sargassum wightii was collected from Taqwa Bay, along the West Mole (Latitude 6° 392 N Longitude 3° 402 E), near Lagos harbour, Nigeria. The specimen was identified at the Phycology unit of the Department of Marine sciences, University of Lagos and a representative specimen was kept for reference purposes. They were washed thoroughly with clean water to remove dirt. *S. wightii* plant was air-dried for three days then pulverized using a mill. The pulverized plant material was packed using a Ziploc bag and stored at room temperature until further use.

Solvent Extraction

Firstly, 200 ml of 95 % ethanol was added to 20 g of powdered *S. wightii* in a 250 ml conical flask and constantly agitated for 72 h. The mixture was sieved using a filter paper and residue was re-extracted. The filtrate was obtained, combined and evaporated to dryness in a hot air oven at 60^{o}C *S. wightii* extract (SWE) was stored in a stoppered bottle at 4^{o}C until further use.

Qualitative and Quantitative Phytochemistry

The extract obtained from *S. wightii* was analysed for the absence or presence of alkaloids, tannins, phlobatannins, saponins, phenols, glycosides, flavonoids, steroids and terpenoids using standard procedures (Harborne, 1998).

In vitro antioxidative potential of SWE

The antioxidative capacity of SWE was initially evaluated using three in vitro methods as earlier described by Thaipong *et al*., (2006). These includes 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical scavenging assay, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation decolourization assay and Ferric reducing antioxidant power (FRAP) assay. Experiments were in triplicate and ascorbic acid was used as the reference compound.

Experimental diet composition

Experimental diets with estimated crude protein and lipid levels of 37 % and 10 % respectively, were supplemented with SWE at various graded levels as shown in Table 1. They include SWE-0 (control (without *S. wightii* extract)), SWE-0.25 (0.25 %), SWE-0.5 (0.5 %) and SWE-1.0 (1.0 %). All ingredients to prepare the basal diet were procured from Agege local market, Lagos state, Nigeria. The ingredients were milled separately, measured, mixed proportionally and thoroughly. Hot water was added to the compounded feed to aid starch gelatinisation and the homogeneous mixture was pelleted at the fish feed processing unit of the Department of Marine Sciences, University of Lagos, Nigeria, using a feed pelletizer fitted with a 2 mm die. The feeds were air-dried, packed in sealed labelled Ziploc bags and stored at 4^{0}C until further use.

Experimental conditions

A total of four hundred *Clarias gariepinus* juveniles were procured from a reputable fish farm (Ak-Marrash fish farm, Egbe, Lagos state, Nigeria.) and conveyed in a 50 L gallon with constant aeration to the wet laboratory of the Department of Marine Sciences, University of Lagos. Acclimatization of the fish

Table 1: The composition of experimental diet

Ingredients %	SWE-0	SWE-0.25	SWE-0.5	SWE-1
Fish meal	20	20	20	20
Soybean meal	25	25	25	25
Groundnut cake	25	25	25	25
Maize	10	9.75	9.5	9
Noodle waste	15	15	15	15
Palm oil	4	4	4	4
Premix	0.5	0.5	0.5	0.5
Dicalcium Phosphate	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1
SWE	0	0.25	0.5	1.0
Total	100	100	100	100
Crude Protein (%)	37.29	37.27	37.24	37.19
Crude Lipid (%)	10.14	10.13	10.12	10.10

SWE = *S. wightii* extract

was carried out in 800 L rearing tank and fed with the basal diet (SWE-0) for a period of 14 days. After acclimatization, one hundred and twenty fish with initial weight of 14.50 ± 0.15 g per fish were randomly allocated in plastic tanks at the rate of ten fish per tank and three replicates for each experimental group. Each experimental group was fed with their assigned diet thrice daily (8:00 am, 1:00 pm, 6:00 pm (WAT)) to apparent satiation for 28 days. Water exchanged was done every other day with dechlorinated water from borehole to maintain good water quality. The dissolved oxygen ranged from 5.0 to 6.0 mg/L, pH ranged from 7.0 to 8.1 and temperature ranged from 27.9 °C to 28.7 °C all through the experimental period.

Growth and Nutrient Utilization Study

The growth performance and nutrient utilization indices were calculated following Bekhan *et al.* (2006).

$$\text{Mean weight gain (MWG, g)} = \text{Final body weight (g)} - \text{Initial body weight (g)} \quad (1)$$

$$\text{Specific growth rate (SGR, \%/day)} = \frac{(\text{Ln}\,final\ body\ weigth - \text{Ln}\,initial\ body\ weight)}{number\ of\ days} \times 100 \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{Feed\ consumed\ in\ dry\ mass\ (g)}{Mean\ weight\ gain\ (g)} \quad (3)$$

$$\text{Protein efficiency ratio (PER)} = \frac{Mean\ weight\ gain\ (g)}{Protein\ Intake\ (g)} \quad (4)$$

$$\text{Where Protein intake (PI)} = Feed\ intake \times Protein\ content\ of\ feed \quad (5)$$

Blood Collection for Laboratory Studies

Fish feeding was stopped 24 h before sampling; blood was collected from the caudal vein of randomly selected fish using a 2 ml syringe. The blood was immediately put into EDTA and plain bottles for haematological and biochemical analysis respectively. For tissue collection, fish was sacrificed, and the harvested liver was transferred to plain bottles containing 2 ml of ice-cold buffer (Dulbecco's phosphate-buffered saline (DPBS)). The tissues were homogenized in DPBS, and homogenates were centrifuged (4^{0}C, 14 000 rpm, 10 min) to obtain the supernatant which was aliquoted and stored at -20 ! till further analysis.

Haematological Analysis

Red blood cells (RBCs) and white blood cells (WBCs) were counted using Neubaeur haemocytometer. Haemoglobin concentration was measured by the

cyanomethaemoglobin spectrophotometry method while Lymphocytes and granulocytes were measured by Giemsa staining method and identified under light microscopy.

Biochemical Analysis

Total protein, albumin, triglycerides, cholesterol, HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), alanine aminotransferase and alkaline phosphatase were analysed at the research laboratory of the Department of Clinical Chemistry, University of Lagos using a biochemical analyser (Erba Mannheim XL 200).

Determination of hepatic antioxidants responses and Malondialdehyde (MDA) levels

Superoxide dimustase (SOD) activity was measured according to Misra & Fridovich (1972) method. Catalase activity was evaluated using the colorimetric assay previously described by Goth *et al*. (1991). Glutathione-S-transferase (GST) activity was determined following the procedures earlier described by Habig *et al*. (1974). The concentration of reduced glutathione (GSH) in hepatic tissue was measured according to Jollow *et al*. (1980) method. Malondialdehyde (MDA) level in tissue homogenates was determined following the procedures earlier described by Nagababu *et al*. (2010).

Enzyme-Linked Immunosorbent assay (ELISA) for determination of IL-6 and TNF-á

The tissue homogenate supernatant was analysed for levels of TNF-á (Tumor Necrosis Factor alpha), IL-1â (Interleukin-1 beta) and IL-6 (Interleukin-6) using respective ELISA kits (BioLegend, USA) with a sensitivity limit of 4 pg/ml. The assay was carried out following the manufacturer's protocols and absorbance read at 450 nm using a LT-4500 microplate reader. The concentration of TNF-á, IL-1â and IL-6 from the hepatic homogenates were extrapolated from the standard curves of TNF-á, IL-1â and IL-6 and expressed as pg/ml.

Statistical analysis

Data are expressed as Mean ± SD of three replicates (n = 3) and were applicable, subjected to one-way ANOVA followed by post-hoc Duncan's multiple comparison test of means ($p < 0.05$). Means were considered statistically significantly different when $P < 0.05$. Statistical analysis was carried out using SPSS (version 22.0).

RESULTS AND DISCUSSION

Phytochemical constituents and *in vitro* antioxidant potential of SWE

There are increasing number of research with similar conclusions that plant extracts are suitable alternatives with great potential to lessen the usage of synthetic chemicals (mainly antibiotics) in aquaculture due to their associated risks (Gabriel, 2019). As shown in Table 2, SWE contains relatively high amount of the tested phytochemicals such as alkaloid, tannins, saponin, phenol, glycoside and flavonoid. Similar phytochemicals have been reported to be present in other brown seaweed species (Chandini *et al*., 2008; Marimuthu *et al*., 2012). As shown in Figure 1, 2,2-diphenyl-1- picrylhydrazyl (DPPH) scavenging activities of SWE was comparable to ascorbic acid. The inhibition percentage of DPPH free radicals increased with higher concentration of the SWE. At 100 µg/ml of SWE the inhibition was at 90% while the inhibition capacity of ascorbic acid was 87%. As shown in Figure 2, the ferric ion-reducing power of SWE increased in a concentration-dependent manner and was comparable to ascorbic acid. Furthermore, Figure 3 shows that SWE exhibited moderate ABTS scavenging activ-

Table 2: Phytochemical constituents of *S. wightii* extract

Tests	(mg/100g)
Alkaloid	32.22 ± 0.03
Tannin	40.26 ± 0.12
Phlobatannin	26.34 ± 0.01
Saponin	44.50 ± 0.57
Phenol	32.41 ± 0.40
Cardiac glycoside	38.10 ± 0.01
Flavonoid	21.36 ± 0.01
Steroid	20.36 ± 0.03
Terpenoid	41.01 ± 0.06

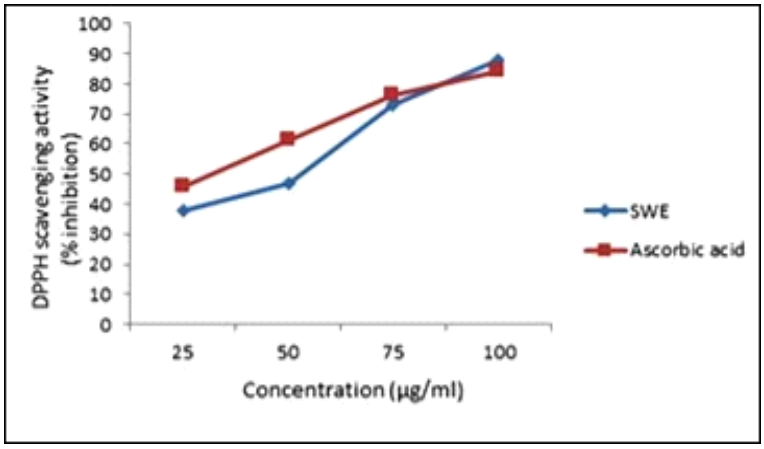

Figure 1. DPPH scavenging activity of *S. wightii* and ascorbic acid.

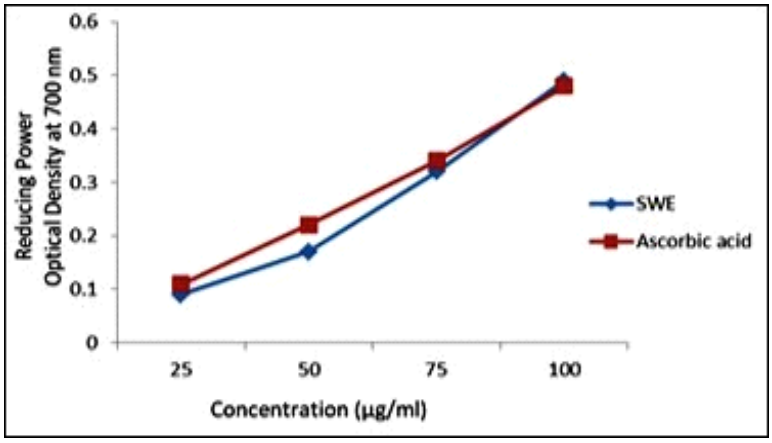

Figure 2. Ferric ion reducing power (FRAP) of *S. wightii* extract and ascorbic acid.

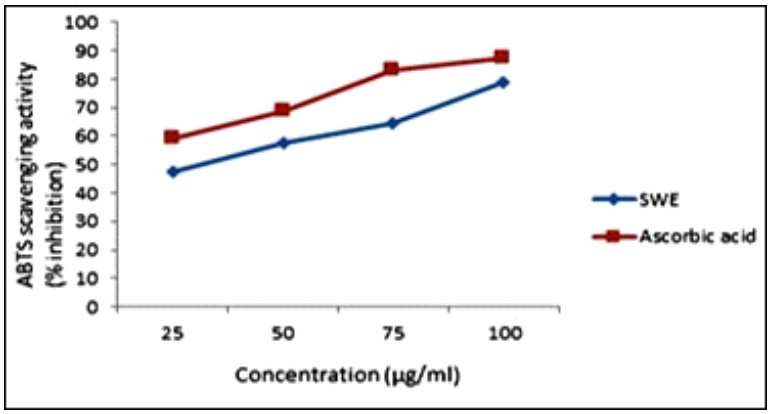

Figure 3. ABTS radical scavenging activity of *S. wightii* extract and ascorbic acid.

ity in a concentration-dependent manner and was comparable to ascorbic acid. As observed in this study, SWE exhibited the potent capacity to scavenge free radicals generated by the *in vitro* assays and therefore substantiates the fact that seaweeds contain bioactive compounds with high antioxidant activity. The antioxidant capacity of brown seaweeds has been attributed to the presence of tannins such as phlorotannin, as they can function as hydrogen and electron donors (Ahn *et al*., 2004). Furthermore, Abdelhamid *et al*. (2018) reported that phlorotannin-rich extracts from brown seaweeds displayed strong antioxidant activities against DPPH and ABTS free radicals.

Dietary effects of SWE on growth performance and nutrient utilization

The growth and nutrient utilization parameters of *C. gariepinus* juveniles fed dietary *S. wightii* extract are shown in Table 3. There was no significant difference ($p > 0.05$) in the mean weight gain across all experimental group. However, feed conversion ratio

(FCR) was lowest in fish fed control diet (SWE-0) which was significantly differently ($p<0.05$) from other experimental groups. Amongst fish fed SWE diet, SWE-1.0 had the lowest FCR and was significantly different ($p<0.05$) from SWE-0.25 and SWE-0.5. Protein efficiency ratio (PER) was significantly higher ($p<0.05$) for SWE-0 group than other experimental groups. Similarly, Schleder *et al*. (2018) had earlier described the non-significant effects of dietary administration of the brown seaweeds *S. filipendula* and *Undaria pinnatifida* on *Litopenaeus vannamei* growth performance. Contrarily, Ashour *et al*. (2020) reported enhanced growth and feed utilisation of *Oreochromis niloticus* fed diets supplemented with seaweed extracts. It has been postulated that the blends of phytogenic compounds might have an antagonistic effect on growth response in fish, and these could be due to high concentrations of phytochemicals with unpleasant taste and/or smell in the mixture (Drewnowski & Gomez-Carneros 2000; Aanyu *et al*., 2020).

Table 3. Growth performance and nutrient utilisation of *C. gariepinus* fed *S. wightii* extract at various inclusion levels.

PARAMETERS	SWE-0	SWE-0.25	SWE-0.5	SWE-1.0
IBW(g)	14.43 ± 0.06^{a}	14.50 ± 0.00^{a}	14.47 ± 0.06^{a}	14.43 ± 0.06^{a}
FBW(g)	40.68 ± 1.21^{a}	35.88 ± 2.14^{a}	36.21 ± 2.61^{a}	37.96 ± 4.24^{a}
MWG	26.22 ± 1.24^{a}	21.40 ± 2.11^{a}	21.78 ± 2.70^{a}	23.54 ± 4.19^{a}
FI(g)	31.98 ± 1.75^{a}	38.53 ± 4.48^{b}	34.50 ± 1.99^{ab}	33.90 ± 0.85^{ab}
FCR	1.20 ± 0.10^{a}	1.57 ± 0.21^{b}	1.57 ± 0.12^{b}	1.33 ± 0.23^{ab}
SGR(%/day)	2.94 ± 0.14^{a}	2.60 ± 0.17^{a}	2.63 ± 0.22^{a}	2.77 ± 0.32^{a}
PI(g)	12.78 ± 0.70^{a}	15.41 ± 1.80^{b}	13.80 ± 0.80^{ab}	13.56 ± 0.34^{ab}
PER	2.07 ± 0.15^{b}	1.57 ± 0.15^{a}	1.63 ± 0.12^{ab}	1.93 ± 0.38^{ab}

Values along row with different superscripts are statistically different ($p < 0.05$). Mean weight gain (MWG); Initial body weight (IBW); Final body weight (FBW); Specific growth rate (SGR); Relative weight gain (RWG); PER = Protein efficiency ratio; Feed conversion ratio (FCR); Protein intake (PI); Feed intake (FI)

Dietary effects of SWE on haemato-biochemical indices

An accurate method for determining the physiological and health status of fish is the study of the haemato-biochemical indicators which could reflect the effects of feed supplements and additives (Fazio, 2019). The effects of SWE on *C. gariepinus* haemato-biochemical indices are presented in Table 4. Dietary SWE significantly increased ($p<0.05$) Haemoglobin, lymphocytes, WBC and RBC counts in a dose-dependent manner in *C. gariepinus*. However, dietary SWE did not cause a significant difference ($p>0.05$) in serum protein, albumin, triglyceride, LDL-C and ALT levels. Cholesterol, HDL-C and ALP significantly increased ($p<0.05$) as the dietary inclusion level of SWE increases. The dietary administration of SWE mediated increased haemoglobin leukocytes and erythrocytes levels which are indicators of improved health status of experimental fish (Esmaeili *et al*. 2021). In line with this finding, Zeraatpisheh *et al*. (2018) had reported enhanced haematological indices in *Oncorhynchus mykiss* fed diets containing brown seaweed extract. It is proposed that seaweed extracts rich in polyphenols could induce erythropoiesis and leucopoiesis processes, thereby contributing to the competency of the immune system (Bulfon *et al*., 2013; Ibe *et al*., 2022). Furthermore, dietary SWE did not alter serum protein, albumin, triglycerides and LDL-C levels but significantly increased cholesterols and HDL-C. Increase in HDL-C is an indicator that excessive production of cholesterol will be expelled thereby preventing the occurrence of atherosclerosis (Fikenzer *et al*., 2018). Also, serum metabolic enzymes such as ALP and ALT reflect the functional status of the liver and indicators of liver injury (Ingawale *et al*. 2014). As observed, the inclusion of SWE in *C. gariepinus* diet did not alter ALT level, however higher inclusion levels of SWE (SWE-0.5 and SWE-1.0) caused significant increase in ALP level in the fish serum. Similarly, Pratheepa *et al*. (2010) recorded an elevation in serum ALP level of *Cyprinus carpio* fed diets supplemented with *Aegle marmelos* leaf extract.

Hepatic antioxidants responses and MDA levels of *C. gariepinus* fed SWE supplemented diets

The antioxidant system combats free radicals and therefore prevents the oxidation of cellular components such as lipids, proteins and nucleic acids (Valko *et al*., 2007; Zhang *et al*., 2019). As shown in Table 5, GST, SOD and MDA levels were not significantly different across all diets. However, experimental group fed *S. wightii* extract had significantly higher ($p<0.05$) glutathione level and catalase activity in liver tissue when compared to SWE-0 group. Fish fed diets supplemented with SWE had undisrupted redox homeostasis and enhanced catalase activities, suggesting higher

Table 4. Haemato-biochemical indices of *C. gariepinus* fed of *S. wightii* extract.

Parameters	SWE-0	SWE-0.25	SWE-0.5	SWE-1.0
WBC (10^3/ µl)	125.53 ± 3.76^a	139.63 ± 3.27^b	151.97 ± 4.56 ^{c}	161.50 ± 4.29 ^{d}
LYMPH (%)	0.43 ± 0.15^a	0.47 ± 0.06^a	1.20 ± 0.10^b	1.23 ± 0.21^b
GRAN (%)	76.60 ± 4.69^a	73.77 ± 2.27^a	84.13 ± 11.49^a	81.43 ± 9.69^a
HB (g/dl)	8.53 ± 1.54^a	10.33 ± 0.21^b	12.23 ± 0.58^c	12.60 ± 0.62^c
RBC (10^6/µl)	2.12 ± 0.37^a	2.71 ± 0.37^b	2.98 ± 0.23^{bc}	3.16 ± 0.12^c
PRO (g/l)	49.45 ± 7.37^a	51.10 ± 9.01^a	43.23 ± 3.53^a	45.10 ± 4.16^a
ALB (g/l)	16.47 ± 3.29^a	16.30 ± 3.73^a	12.80 ± 1.13^a	14.27 ± 2.15^a
TRIG (mmol/l)	1.83 ± 0.12^a	1.87 ± 0.06^a	1.93 ± 0.23^a	1.93 ± 0.45^a
CHOL (mmol/l)	1.43 ± 0.23^a	1.47 ± 0.32^a	1.90 ± 0.20^{ab}	2.00 ± 0.17^b
HDL-C (mmol/l)	1.43 ± 0.23^a	1.47 ± 0.32^a	1.90 ± 0.20^{ab}	2.00 ± 0.17^b
LDL-C (mmol/l)	0.43 ± 0.06^a	0.60 ± 0.10^a	0.60 ± 0.06^a	0.63 ± 0.15^a
ALP	15.03 ± 1.59^a	14.83 ± 1.96^a	23.37 ±1.95^b	22.53 ± 1.00^b
ALT	38.90 ± 7.30^a	43.00 ± 11.96^a	41.33 ± 4.71^a	38.30 ± 0.95^a

Data expressed as mean ± SD, values along row with the same superscript were not significant different ($p > 0.05$). WBC- white blood cells, LYMPH- Lymphocytes, GRAN- Granulocytes, HB- haemoglobin, RBC- red blood cells, PRO- Protein, ALB- Albumin, MCV- mean corpuscular volume. ALB- albumin, CHOL- cholesterol, HDL-C- high-density lipoprotein cholesterol, TRIG- triglycerides, LDL-C- low-density lipoprotein cholesterol, ALP- Alkaline phosphatase, ALT-alanine transaminase.

Table 5: Effect of SWE on hepatic antioxidants and MDA levels of *C. gariepinus*

Parameters	SWE-0	SWE-0.25	SWE-0.5	SWE-1.0
SOD	0.49 ± 0.03^b	0.51 ± 0.02^b	0.48 ± 0.05^b	0.31 ± 0.04^a
CAT	13.53 ± 0.76^a	15.70 ± 0.41^{bc}	14.74 ± 0.61^{ab}	16.57 ± 1.06^c
GSH	3.80 ± 0.36^a	4.93 ± 0.58^{ab}	5.27 ± 0.64^b	6.13 ± 0.80^c
GST	0.45 ± 0.07^a	0.53 ± 0.04^a	0.51 ± 0.05^a	0.44 ± 0.06^a
MDA	0.77 ± 0.01^a	0.81 ± 0.14^a	0.82 ± 0.08^a	0.80 ± 0.10^a

Superoxide dismutase (SOD); Catalase (CAT); Reduced glutathione (GSH); Glutathione-S-Tranferase (GST); (E) malondialdehyde (MDA) levels. Data are presented as mean ± S.D (n = 3) and different superscript on top bars indicate the significant differences between experimental groups ($p < 0.05$).

Table 6. Immune responses of *C. gariepinus* fed graded levels of *S. wightii* extract.

Parameters	SWE-0	SWE-0.25	SWE-0.5	SWE-1.0
TNFα	5.09 ± 0.53^a	8.62 ± 0.45^b	7.01 ± 0.85^b	8.58 ±1.34^b
IL-1β	23.18 ± 3.46^b	27.19 ± 0.28^c	23.62 ± 1.85^{bc}	15.08 ± 0.89^a
IL-6	23.30 ± 2.33^b	26.59 ± 2.95^b	32.48 ± 1.58^c	9.99 ± 0.68^a

Values along rows with the same superscript are not significantly different ($p > 0.05$).

efficiency to neutralise hydrogen peroxides and impede lipid peroxidation. This implies that SWE had the capacity to scavenge free radicals and promoted antioxidative activities *in vitro* and *in vivo* respectively.

Effect of dietary SWE on *C. gariepinus* immune responses

Cytokines are small proteins that serve as signalling molecules, allowing cells of the immune system to communicate with each other. TNF-á contributes to the limitation of pathogen multiplication and faster clearance in the liver (Zou *et al*., 2016), IL-6 mediates physiological functions (Hodge *et al*., 2005) while IL-1â plays important role in regulating monocytes and macrophages (Corripio-Miyar *et al*., 2007). As presented in Table 6, the supplementation of *C. gariepinus* diets with SWE caused significant increases the production of IL-1â, IL-6 and TNF-á in hepatic tissue. TNF-á production significantly increased ($p < 0.05$) in fish fed SWE diets when compared to the control (SWE-0), however, there was no significant different amongst SWE dietary groups. IL-1â was significantly different ($p < 0.05$) across all dietary groups with SWE-0.25 inducing the highest production (27.19 ± 0.28)

of IL-1â, followed by SWE-0.5 (23.62 ± 1.85) while the lowest production of IL-1â was in fish fed SWE-1.0 diet. Also, IL-6 increases in SWE-0.5 dietary group and was significantly different ($p<0.05$) from other dietary groups. As observed, SWE induced the production of the cytokines in *C. gariepinus*, suggesting improved immunity. Lee *et al*., (2020) reported that extract of the brown seaweed *Sarcodia suiae* up-regulated the expression of IL-1â and TNF-á in *O. niloticus*. SWE capacity to stimulate immune responses may be due to the occurrence of glycosides and alkaloids, which are found to be major bioactive compounds responsible for the immunostimulatory properties of plant extracts (Galina *et al*., 2009).

CONCLUSION

S. wightii extract is a rich source of phenolic compounds with great potentials to improve the physiological status of cultured fish species. This study indicated that the inclusion of *S. wightii* extract in *C. gariepinus* diet stimulated immune responses and improved antioxidant activities of *C. gariepinus*. Therefore, SWE possesses potential for application as feed additive in the aquaculture industry.

ACKNOWLEDGEMENTS

The authors appreciate the University of Lagos Central Research Committee (CRC 2021/03) for funding this research project and Mr Mensah Peter for providing technical assistance.

REFERENCES

Aanyu, M., Betancor, M. B. & Monroig, O. (2020). The effects of combined phytogenics on growth and nutritional physiology of Nile tilapia *Oreochromis niloticus*. *Aquaculture*, 519, 734-767.

Abdelhamid, A., Jouini, M., Bel Haj Amor, H., Mzoughi, Z., Dridi, M., Ben Said, R., & Bouraoui, A. (2018). Phytochemical analysis and evaluation of the antioxidant, anti-inflammatory, and antinociceptive potential of phlorotannin-rich fractions from three Mediterranean brown seaweeds. *Marine Biotechnology*, 20, 60-74.

Afreen, A. B., Rasool, F., & Fatima, M. (2023). Bioactive properties of brown seaweed, *Sargassum wightii* and its nutritional, therapeutic potential and health benefits: A review. *Journal of Environmental Biology*, 44(2), 146-158.

Ahn, M. J., Yoon, K. D., Min, S. Y., Lee, J. S., Kim, J. H., Kim, T. G., & Kim, J. (2004). Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from the brown alga *Ecklonia cava*. *Biological and Pharmaceutical Bulletin*, 27(4), 544–547

Almeida, Â., Solé, M., Soares, A. M., and Freitas, R. (2020). Anti-inflammatory drugs in the marine environment: Bioconcentration, metabolism and sub-lethal effects in marine bivalves. *Environmental Pollution*, *263*, 114-122.

Ashour, M., Mabrouk, M. M., Ayoub, H. F., El-Feky, M. M., Zaki, S. Z., Hoseinifar, S. H., ... & Goda, A. M. S. (2020). Effect of dietary seaweed extract supplementation on growth, feed utilization, hematological indices, and non-specific immunity of Nile Tilapia, Oreochromis niloticus challenged with Aeromonas hydrophila. *Journal of Applied Phycology*, 32, 3467-3479.

Baleta, F.N., Laureta, L.V., Apines-Amar, M.J., Padilla, P.I., Quinitio, G.F. (2011). Biological activity of extracts of *Sargassum oligocystum* (Magnaye) against aquaculture pathogenic bacteria. *Israeli Journal of Aquaculture-Bamidgeh*, 63, 667-671

Bekhan, S., Dogankaya, L., & Cakirogullari, G. C. (2006). Growth and body composition of European catfish (Silurus glanis L.) fed diets containing different percentages of protein. *Israeli Journal of Aquaculture-Bamidgeh*, 58, 1-4

Bolaños, J.M., Baleta, F.N., & Cairel, J.D. (2017). Antimicrobial properties of *Sargassum* spp. (Phaeophyceae) against selected aquaculture pathogens. *International Journal of Current Microbiology and Applied Sciences*, 6, 1024-1037

Bulfon, C., Volpatti, D., & Galeotti, M. (2013). Current research on the use of plant-derived products on farmed fish. *Aquaculture Research*, 46, 1–39.

Chandini, S. K., Ganesan, P., & Bhaskar, N. (2008). In vitro antioxidant activities of three selected brown seaweeds of India. *Food chemistry*, 107(2), 707-713.

Corripio-Miyar, Y., Bird, S., Tsamopoulos, K., & Secombes, C. J. (2007). Cloning and expression analysis of two pro-inflammatory cytokines, IL-1â and IL-8, in haddock (*Melanogrammus aeglefinus*). *Molecular Immunology*, 44(6), 1361-1373

Dauda, A. B., Natrah, I., Karim, M., Kamarudin, M. S., & Bichi, A. U. H. (2018). African catfish aquaculture in Malaysia and Nigeria: Status, trends and prospects. *Fisheries and Aquaculture Journal*, 9(1), 1-5.

Deepak, P.V., Amutha, R., Birundha, R., Sowmiya, C., Kamaraj, V.B., Balasubramani, G., Aiswarya, D., Arul, D., Perumal, P. (2018). Facile green synthesis of nanoparticles from brown seaweed *Sargassum wightii* and its biological application poten-

tial. *Advances in Natural Sciences, Nanosciences & Nanotechnology*, 9, 1-10

Deepika, R.C. (2017). Fucose-containing sulfated polysaccharides from sargassum wight ii: extraction technology and anticancer activity assessment. *International Journal of Pharmaceutical Chemistry & Biological Sciences*, 7(3), 279-284

Devi, K.N., Kumar, T.T.A., & Balasubramanian, T. (2014). Antibacterial and antioxidant effects from seaweed, Sargassum *wightii* (Greville, 1848) against marine ornamental fish pathogens. *Journal of Coast Life & Medicine*, 2, 773-783

Drewnowski, A., & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *American Journal of Clinical Nutrition*, 76 (6): 1424-1435.

Esmaeili, N. (2021). Blood performance: a new formula for fish growth and health. *Biology*, 10(12), 1236.

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture 500: 237-242.

Fikenzer, K., Fikenzer, S., Laufs, U., & Werner, C. (2018). Effects of endurance training on serum lipids. *Vascular Pharmacology*, 101, 9–20

Gabriel, N.N. (2019). Review on the progress in the role of herbal extracts in tilapia culture. *Cogent Food & Agriculture*, 5(1), 16-19

Galina, J., Yin, G., Ardo, L., & Jeney, Z. (2009) The use of immunostimulating herbs in fish. An overview of research. *Fish Physiology & Biochemistry*, 35, 669-676

Generaliæ M. I., Skroza, D., Šimat, V., Hamed, I., Èagalj, M., & Popoviæ, P. (2019). Phenolic content of brown algae (Pheophyceae) species: Extraction, identification, and quantification. *Biomolecules*, *9*(6), 244.

Giddings, L. A., Newman, D. J., Giddings, L. A., & Newman, D. J. (2015). *Bioactive compounds from marine extremophiles*. Springer International Publishing New York, U.S.A. pp. 1-124.

Goth, L. (1991). A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, 196(2-3), 143-151

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249(22), 7130-7139.

Harborne, J.B. (1998). Phytochemical Methods: A guide to modern techniques of plant analysis 3rd edn. Chapman and Hall, New York. pp. 1-150

Hodge, D. R., Hurt, E. M., & Farrar, W. L. (2005). The role of IL-6 and STAT3 in inflammation and cancer. *European Journal of Cancer*, 41(16), 2502-2512.

Ibe, O. E., Akuodor, G. C., Elom, M. O., Chukwurah, E. F., Ibe, C. E., & Nworie, A. (2022). Protective effects of an ethanolic leaf extract from Ficus capensis against phenylhydrazine induced anaemia in Wistar rats. *Journal of Herbmed Pharmacology*, 11(4), 483-489

Ingawale D. K., Mandlik S. K., & Naik S. R. (2014). Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): a critical discussion. *Environmental Toxicology and Pharmacology,* 37(1), 118–133.

Jollow, D. J., Mitchell, J. R., Zampaglione, N. A., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, 11(3), 151-169.

Kumar, C. S., Ganesan, P., Suresh, P. V., & Bhaskar, N. (2008). Seaweeds as a source of nutritionally beneficial compounds-a review. *Journal of Food Science and Technology*, 45(1), 1.

La Barre, S., Potin, P., Leblanc, C., & Delage, L. (2010). The halogenated metabolism of brown algae Phaeophyta: its biological importance and its environmental significance. *Marine Drugs*. 8(4), 988-1010.

Lee, P. T., Wen, C. M., Nan, F. H., Yeh, H. Y., & Lee, M. C. (2020). Immunostimulatory effects of Sarcodia suiae water extracts on Nile tilapia Oreochromis niloticus and its resistance against Streptococcus agalactiae. *Fish and Shellfish Immunology*, 103, 159-168.

Marimuthu, J., Essakimuthu, P., Narayanan, J., Anantham, B., Tharmaraj, R. J., & Arumugam, S. (2012). Phytochemical characterization of brown seaweed Sargassum wightii. *Asian Pacific Journal of Tropical Disease*, 2, 109-113.

Mattio, L., & Payri, C. (2011). 190 years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. *Botanical Review*, 77, 31–70

Mbokane, E. M., Mbokane, L. M., Motimele, S. S., & Hlophe-Ginindza, S. N. (2022). Successes and Challenges of Catfish Farming in the Small-Scale Industry in Southern Africa. In: *Catfish-Advances, Technology, Experiments*. IntechOpen. 96 pp

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170-3175.

Moghadamtousi, S. Z., Nikzad, S., Abdul Kadir, H., Abubakar, S., & Zandi, K. (2015). Potential antiviral agents from marine fungi: An overview. *Marine drugs*. 13(7), 4520-4538.

Nagababu, E., Rifkind, J.M., Boindala, S., & Nakka, L. (2010). Assessment of antioxidant activity of eugenol *in vitro* and *in vivo*. In: Free Radicals and Antioxidant Protocols. Human Press. pp 165-180.

Pratheepa, V., Ramesh, S., & Sukumaran, N. (2010). Immunomodulatory effect of *Aegle marmelos* leaf extract on freshwater fish *Cyprinus carpio* infected by bacterial pathogen *Aeromonas hydrophila*. *Pharmaceutical Biology*, 48(11), 1224-1239

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plants extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433, 50-61

Schleder, D. D., Peruch, L. G. B., Poli, M. A., Ferreira, T. H., Silva, C. P., Andreatta, E. R., ... do Nascimento Vieira, F. (2018*).* Effect of brown seaweeds on Pacific white shrimp growth performance, gut morphology, digestive enzymes activity and resistance to white spot virus. *Aquaculture,* 495, 359–365. doi:10.1016/j.aquaculture.2018.06.020

Shibata, T., Ken, F., Kohki, N., Kuniko, Y., & Takashi, N. (2002). "Inhibitory activity of brown algal phlorotannins against hyaluronidase". *International Journal of Food Science and Technology*, 37(6), 703-709

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19, 669–675. doi: 10.1016/j.jfca.2006.01.003.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M. & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, 39(1), 44-84

Zeraatpisheh, F., Firouzbakhsh, F., & Khalili, K. J. (2018). Effects of the macroalga *Sargassum angustifolium* hot water extract on hematological parameters and immune responses in rainbow trout (*Oncohrynchus mykiss*) infected with *Yersinia rukeri*. *Journal of Applied Phycology*, 30, 2029-2037

Zhang, B., Li, C., Wang, X., Zhou, H., Mai, K., & He, G. (2019). The effects of dietary *Eucommia ulmoides* oliver on growth, feed utilization, antioxidant activity and immune responses of turbot (*Scophthalmus maximus* L.). *Aquaculture Nutrition*, 25(2), 367-376

Zou, J., & Secombes, C.J. (2016). The function of fish cytokines. *Biology*, 5(2):23-27

