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ANTIOXIDANT ACTIVITY AND CHEMICAL COMPOSITION OF *Clarias gariepinus* FROM NATURE AND CAPTIVITY

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ABSTRACT

Gradual shift from eating healthy to unhealthy food is a major contributor to development of non-communicable diseases . This study compared the antioxidant activity and chemical composition of wild and cultured *Clarias gariepinus*. A total of 10 fish each were collected from both environments. Nutrient composition, antioxidant activity, fatty acid and heavy metals load of the samples were determined using standard methods. Significant difference was observed in the proximate composition (crude protein (CP), Ether Extract (EE) and Nitrogen free extract in both samples, with higher values of CP (54.98 ± 0.66%) and EE (34.17 ± 0.33%) observed in the wild sample. No significant difference was observed in the values for sodium, potassium, phosphorus, calcium, magnesium, zinc and iron. However, manganese (350.93 ppm) was significantly ($P<0.05$) higher in the cultured species. Scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was not statistically different ($P > 0.05$) at 50 mg/ml concentration. Hydrogen peroxide radical scavenging activity was however low in cultured and wild samples (1.27 and 8.12 mg/100 g) respectively. Heavy metals level in both samples were not statistically different ($P>0.05$). It was concluded that the cultured *C. gariepinus* compared favourably with the wild species in their mineral composition, antioxidant activity, and heavy metal content as opposed to local belief.

Keywords: Diseases, cultured and wild *Clarias gariepinus*, produce, consumption, food safety, food security

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INTRODUCTION

Many developing countries, Nigeria inclusive are gradually moving from consuming healthy foods to unhealthy foods due to several factors including poverty. This exposes consumers to several diet linked metabolic diseases including diabetes, hypertension, cancers and so on (Hernández-Ledesma *et al.*, 2017). Fish is usually consumed due to its supply of qualitative proteins to diet. In recent times, scientists are interested in other benefits that may be derived from fish based on its chemical components which may help in ameliorating

some of these metabolic disorders. These includes a majority of human non-communicable diseases e.g. atherosclerosis, diabetes, cancer, inflammatory joint disease, which might have originated from metabolic oxidative reactions (Florence, 1995).

Nutrients and micronutrients obtained from fish and fish waste such as fatty acids peptides, water soluble minerals, enzymes, and oligosaccharides, have high bioactivity (Ngodigha *et al.*, 2017). Senevirathne *et al.*, (2012) reported that these compounds have potential for diverse applications to include antibacterial, antihypertensive, antiviral, antioxidants and anticancer properties.

From the recent past till date, phyto- and zoo- therapy have been harnessed both in folk medicines and in pharmaceuticals (Alves and Rosa, 2005). World Health Organization (WHO) reported that 11% and 8.7% of the 252 essential chemicals used in the production of modern medicines originated from plants and animals respectively (Ngodigha *et al.*, 2017). The medicinal importance of fish can be attributed to the presence of the oil, proteins, and other classes of nutrients in the fish making the product an important raw material for pharmaceuticals and essentially the beneficial omega-3 fatty acids (Durmuş, 2019).

There are however, few researches on the medicinal properties of marine and fresh water fish species in Nigeria. Some documented studies include the review on fin fishes in Yoruba natural healing practices (Sowunmi, 2007), *Channa striata* extract as potential agent for cancer (Buhari *et al.*, 2015), and therapeutic potential of fish by-products (Ngodigha *et al.*, 2017). Further studies on the subject matter are needed especially with the increased human mortality due to communicable and non-communicable diseases globally. This study was therefore designed to evaluate the antioxidant and biochemical composition of the wild and cultured sharp tooth catfish *Clarias gariepinus* as a preliminary study into the medicinal properties of this species

MATERIALS AND METHODS

Collection of experimental fish

Ten *Clarias gariepinus* each were obtained from wild and pond culture. Wild fish samples were bought from fishers in Ero Dam, Osi, Kwara State. Ero Dam in Kwara State has its source from Ero Dam in Ekiti State (Figure 1). Ero Dam is situated at Ikun-Ekiti, Moba Local Government Area of Ekiti State, Nigeria between latitudes 7˚15' and 8˚5'N and longitude 4˚45' and 5˚31'E. Not much of fishing activities is taking place at the dam site. The fish samples were transported in ice cold water in a 50 L plastic keg to the laboratory for analyses.

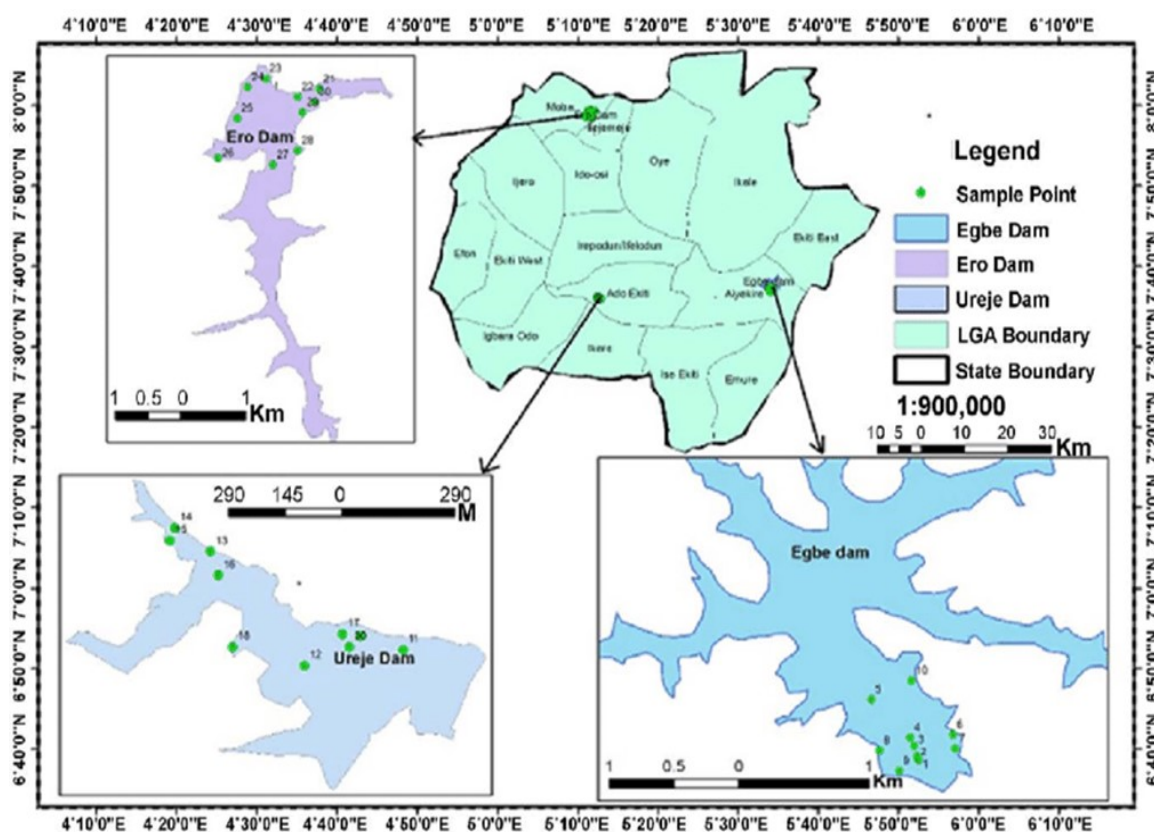

Figure. 1: Map of Ekiti State showing Ero dam

Source: Olagbemide and Owolabi (2019)

Identification of fish sample

Fish samples were identified using identification keys described by Idodo-Umeh (2003).

Preparation of fish sample

The fish samples were weighed, cleaned and oven dried at a temperature of 40 ºC for five (5) days. The muscles of the dried samples were finely milled using an electronic blender (Euro Premium, Model-Cm/l:7398801), kept in airtight container and labelled appropriately for further analyses.

Proximate composition analysis

The proximate analysis of fish samples was carried out using the Association of Analytical Chemist (AOAC, 2005) method. Carbohydrate percentage was thereafter calculated for by subtracting total nutrient quantified from 100.

Mineral profile determination of samples

The preparation of samples for mineral elements analysis followed the Dry ashing method of preparation described by Chapman and Pratt (1962). A 0.5 g of samples were weighed into a 30 ml porcelain crucible, the porcelain was put in a cool muffle furnace and the temperature gradually raised to

550°C. Ashing was then continued for 24 hours. After cooling, ash was melted in 2N HCl (5 ml portion) and stirred thoroughly. Macro minerals were determined using Atomic Absorption Spectrometer (Model number: AA320N). UV-V Spectrophotometer (P determination), EDTA titration for Ca and Mg in which standard solutions (1000 µg / ml) of Na, K, P, Ca, Mg, Zn, Fe and Mn were prepared.

Fatty acid profile determination

Determination of fatty acid was as described in the AOAC (2005) by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler and a flame ionization detector (FID). Helium (1.8 mL min-1) carrier gas was used for separation. A fused silica capillary column (OmegawaxTM-320, 30 m × 0.32 mm i.d.) was used. The column temperature was set to start at 180°C for 20 minutes and afterwards, temperature was raised to 200°C gradually (for every 1 minute, temperature was increased by 1°C). Temperature was sustained at 200°C for another 1 minute and heated again to 220°C at 5°C/minute. Finally, it was held at 220°C for 20 min. A split injector (50:1) at 250°C was used. The FID was also heated at 250°C. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.). Their quantification was made by relating the peak area to the area of an internal standard (methyl tricosanoate).

Retention time and NIST library was used for identification.

Antioxidant Assay

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Testing

The method described by Nithianantham *et al.* (2011) for antioxidant assay against DPPH free radical scavenging was used. One mililitre of various concentrations of the samples in methanol was added to 4 mL of 0.1 mmol L–1 methanolic solution of DPPH. A blank probe was obtained by mixing 4 mL of 0.1 mmol L–1 methanolic solution of DPPH and 200 µL of deionized distilled water (ddH_2O). After 30 minutes of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent (I %) was calculated using the formula:

%Inhibition = 100-((ABS sample – ABS blank)/ ABS control) x100

Hydrogen Peroxide (H_2O_2) Assay

H_2O_2 scavenging activity was assayed for as described by Ahmad *et al.* (2014). Phenol (12 mM) and 4-aminoantipyrene (0.5 mM) were chosen and used for the test as these concentrations led to maximum intensity of the resultant chromophore. The percentage inhibition (% I) of H_2O_2 caused by extracts and standard antioxidants was calculated. Reaction mixture comprising of test sample (fish samples/standard antioxidant; 350 ml), phenol solution (12 mM, 350 ml), 4-aminoantipyrene (0.5 mM, 100 ml), H_2O_2 (0.7 mM, 160 ml) and HRP (1 U/ml) prepared in phosphate buffer (84 mM, pH 7) was incubated at 37°C for 30 minutes. The absorbances of the resulting solutions were measured at 504 nm against reagent blank consisting of phosphate buffer instead of fish samples standard antioxidant and phenol. The control (Gallic Acid) was made out of same reagents except fish sample replaced by phosphate buffer. Interference for the

assay from the fish samples was minimized. Each concentration of fish extract, samples for background subtraction were made using the fish extract with other reagents replacing phenol by phosphate buffer.

Each resulting absorbance value was subtracted from the relevant original absorbance reading. L-Ascorbic acid, gallic acid and tannic acid were used as reference standard antioxidants. The percentage inhibition of hydrogen peroxide was calculated by the equation as described for many antioxidant assays.

%Inhibition =Abs. of control – Abs. of sample /Abs. of control x100%

Heavy Metals

The method described by Association of Official Analytical Chemists (AOAC, 2005) was used for mineral analysis. The samples were ashed at 550°C. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. This was made up to the mark with deionized water. Arsenic, Mercury, Lead, Cadmium, Iron, Nickel, Copper and Chromium were determined using Atomic Absorption Spectrophotometer (AAS PG Instrument Model 990FG). Hg was analysed using ED-XRF Asoma Phonix II. Values obtained were recorded in mg/100 g.

RESULTS

Proximate composition

Crude protein (CP) was higher ($P<0.01$) in the wild fish compared with the cultured sample. NFE showed marked variations in the samples with higher values ($P<0.01$) observed in the cultured sample than in the wild samples (Table 1).

Table 1: Proximate composition (%) of experimental fish

SAMPLE	CP	EE	ASH	DRY MATTER	NFE
CF	52.61 ± 0.10	24.67± 0.17	3.33± 0.67	1.67± 0.17	17.73± 0.50
WF	54.98 ± 0.66	34.17 ± 0.33	3.17 ± 0.17	2.17 ± 0.17	5.52 ± 0.92
t (0.01) Sig.	0.061***	0.001***	0.808	Nil	0.008***

Data are presented are in means± SE of the mean. ***Significant at 1%. CF – Cultured fish, WF – Wild fish, NFE - Nitrogen free extract, EE- Ether extract, CP - Crude protein.

Mineral composition

No statistical variation was observed at 95% probability in the values of Na, K, P, Ca, Zn and Fe. Manganese was significantly different in both samples (Figure. 2).

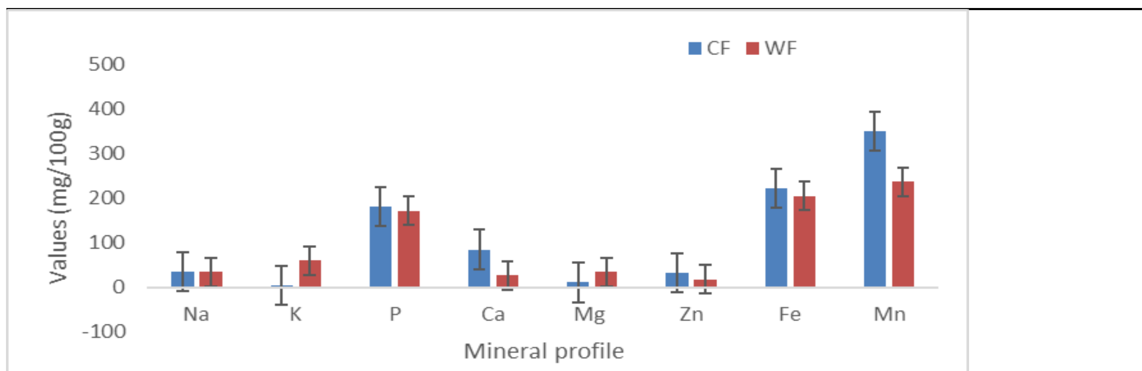

Figure 2: Mineral profile of fish samples. CF – Cultured fish, WF – Wild fish. Na-Sodium, K-Potassium, P-Phosphorus, Ca- Calcium, Mg- Magnesium, Zn- Zinc, Fe-Iron, Mn- Manganese

Fatty Acid Composition

Compounds present were mainly aldehyde, alcohols, dicarboxylic acid, esters and alkenes (Table 2).

Antioxidant activity of experimental fish

Effect of fish samples on DPPH scavenging assay

The cultured and wild *C. gariepinus* muscles showed a high scavenging activity against DPPH concentration dependent (Fig. 3). Between cultured and wild samples, no significant difference was observed at 95% probability at various concentrations, however, cultured sample compared well with the standard antioxidant BHT at higher concentrations (Fig. 3).

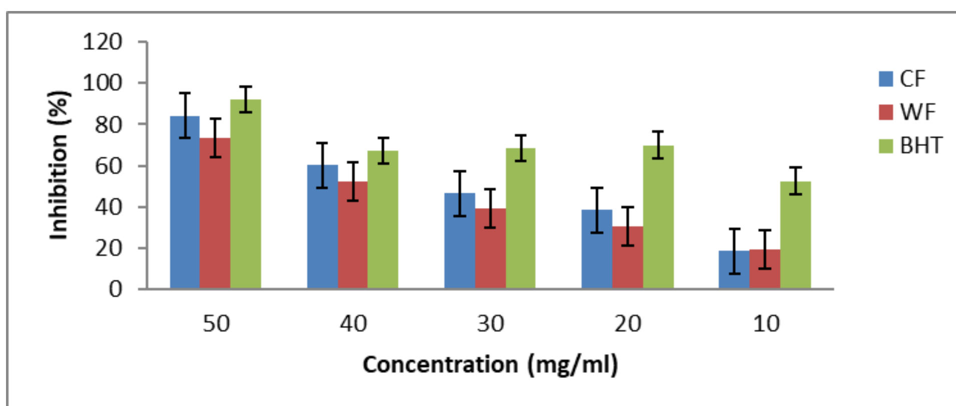

Fig. 3: *2, 2-diphenyl-1-picrylhydrazyl* scavenging activity of the cultured and wild *Clarias gariepinus*

Table 2: Compounds Detected in the Experimental Samples Oil

S/N	COMPOUND	SOURCE	APPLICATIONS	REFERENCE
1	1H-Imidazole 2-ethyl-4-methyl-	WF	Pharmaceutical and medicine manufacturing Plastic material and resin manufacturing	Venkatesan *et al.* (2008); Kumar *et al.*, 2017
2	5,7-Octadien-3-ol 2,4,4,7-tetramethyl-(E)-	WF	NIL	NIL
3	Pyridine 2,6-dichloro-3-nitro-	WF	For the production of herbicides and compounds having analgesic properties	Daily Chronicles (2020)
4	Cyclohexene 4-methylene -1-(1methylethyl)-	WF	Industrial fragrance	Eggersdorfer (2005)
5	(-)-cis-Myrtanyl Acetate	WF	NIL	NIL
6	Bicyclo[2.2.1] heptane	WF	NIL	NIL
7	2-chloro-2, 3,3-trimethyl-	WF	NIL	NIL
8	Cyclononasiloxane, Octadecamethyl	CF	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites	Lassen *et al.* (2005)
9	Cyclohexasiloxane, Dodecamethyl-	CF	Cleaning agents, cosmetics, textile applications, antifungal and as biological resistance to termites	Gascón-Garrido *et al.* (2017)
10	Borane	CF	Cancer therapy and diagnostics and therapy of radionuclide. Also used to ease conditions such as arthritis, osteoporosis, and coronary heart disease in animals	Soriano-Ursúa *et al.* (2014)
11	Ethyl dipropyl-	CF	For the production of other chemicals	NCBI (2020)
12	Fumaric acid	CF	Food additive pharmaceuticals, in the manufacture of polyester resins, polyhydric alcohols and dyes	Fox *et al.* (2012)
13	Dec-4-enyl heptadecyl ester	CF	NIL	
14	2,3-Pentadiene	CF	NIL	

Effect of fish samples on Hydrogen peroxide* (H_2O_2) *radical scavenging activity

There was no statistical variation (P>0.05) between cultured and wild *Clarias gariepinus*. The samples exhibited very low inhibition against H_2O_2 as opposed to the standard gallic acid used (Fig. 4).

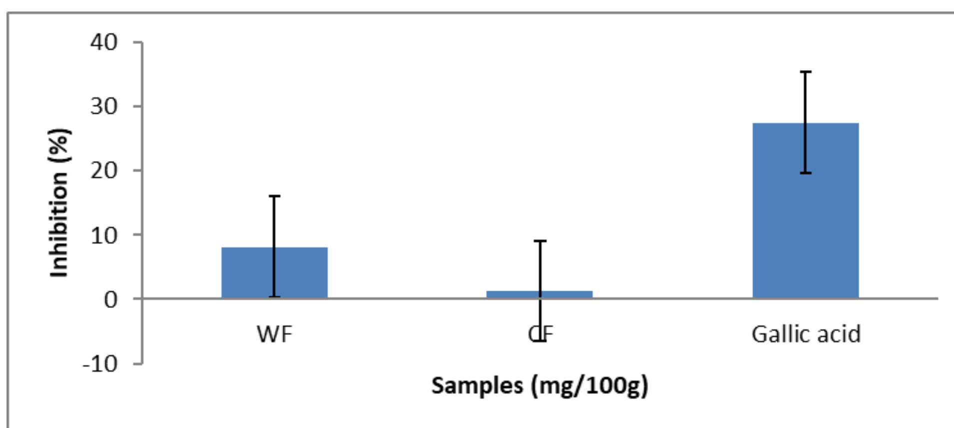

Figure 4: Hydrogen peroxide radical scavenging activity of the cultured and wild *Clarias gariepinus*

Heavy metal composition

No statistical variation (P>0.05) was observed between the level of heavy metals present in cultured and wild samples (Table 3).

Table 3: Heavy Metal Composition of Experimental Fish

Parameters (ppm)	Cultured fish	Wild fish	Permissible limit	Reference
Arsenic	Nil	0.09±0.00	6.0	FAO/WHO (2004)
Cadmium	0.02±0.00a	0.01±0.00a	0.5	FAO (1983)
Chromium	0.16±0.00a	0.08±0.00a	-	FAO/WHO (1989)
Copper	0.04±0.00a	0.03±0.00a	30	FAO/WHO (1989)
Iron	1.24±0.00a	1.27±0.00a	0.5	FAO (2012)
,Mercury	0.67±0.00a	0.67±0.00a	0.500	FAO (2003)
Nickel	0.30±0.00a	0.33±0.00a	0.5-0.6	FAO (2012)
Lead	0.14±0.00a	Hould have a specific 0.10±0.00a	0.400	FAO (2003)

Key: Data are presented in means ± SD of the mean

DISCUSSION

The high level of crude protein in wild fish that compared to cultured ones could be due to the variety of natural food available in the natural environment of the sample (Obaroh *et al*., 2015). High protein content in the samples gives an indication that *C. gariepinus* either from nature or from captivity is a good source of protein and better than some other conventional animal proteins, giving the species a high nutritional and medicinal advantage. The proximate composition of the wild and cultured species which showed significant variation in amount of CP, nitrogen free extract and EE, was similar to the findings of Ukagwu *et al*. (2017) and could be attributed to food availability.

In terms of mineral composition, the values obtained for Na, K, P, Zn and Fe corroborated that of Adelakun *et al*. (2017) who documented that manganese; iron, phosphorus, calcium etc. are present in *C. gariepinus*. Presence of manganese in the fish confers antioxidative functions and therefore health benefits (Li and Yang, 2018). Calcium is known to play a significant role in bone and strong teeth formation (Johnson, 2006), therefore, consumption of the species can play essential role in strengthening bones and teeth. Zinc obtained in both samples indicate that the species, whether from nature or captivity can act as an important immune stimulant (Binghama, 2005). Deng *et al*. (2016) reported similar trend in most of the minerals measured in their study. This was however not the case in the study of Nwali *et al*. (2015) who recorded significant differences in the levels of minerals in both cultured and wild fish species.

The composition of the compounds identified in this study are with varied medicinal uses. These differed from the report of previous researches (Okonji *et al*., 2014; Taiwo *et al*., 2014) on the fatty acid profile of this species either from cultured or wild samples. This may be as a result of differences in the state of oxidation of the samples at the point of analysis and environmental variations (Albert *et al*., 2013; Sullivan Ritter and Budge 2012).

The antioxidant activity of *C. gariepinus* from nature and captivity measured in this study could be attributed to their mineral compositions, which act as co-factors for some antioxidant enzymes, as well as proteins and fatty acid profile of the species. Studies have shown that fish muscle protein and oil exhibit antioxidant activity (Anderson *et al*., 2014; Nazeer and Kulandai, 2012). The finding of this study corroborated previous research on the antioxidant activity of some fish species (Elavarasan *et al*., 2014; Nazeer and Kulandai, 2012).

Heavy metals assessed in the cultured and wild species were within the permissible ranges documented by FAO and WHO with exception of Fe and Hg. Though some metals are important to animal life as they perform known important biological role, however excess or lack of it can result to some level of toxicity thereby hampering some biological processes such as enzyme activities, immune stimulation and much more (Elbeshti *et al*., 2018). The reported levels of metals from the study implies that the samples may not pose any human health risk sequel to ingestion.

The study concludes that cultured *C. gariepinus* compared favourably with the wild species, although, crude protein and ether extract was higher in wild species than cul-

tured. Various fatty acid compounds with varying applications were also obtained from the wild and cultured *C. gariepinus.* The mineral profile of wild and cultured species were not variably different. Wild and cultured *C. gariepinus* demonstrated concentration-dependent free radical (DPPH) and hydroxyl radical ($OH^•$) scavenging activities with contents of heavy metals in the wild and cultured species within the permissible limit recommended by FAO/WHO for fish. The fish samples may therefore confer medicinal values to the consumers, with minimal health risk due to the low levels of heavy metals present in the sampled wild and cultured *C. gariepinus*.

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