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Evaluation of visceral oil from aquaculture african catfish (*Clarias gariepinus*) and giant sea catfish (*Arius gigas*) sold in Uyo, Nigeria

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ABSTRACT

Background: Fish oils serves as valuable medicinal drugs and food supplements globally depending on its quality. Analyses of oil present in the viscera of two commonly consumed fish species in Uyo metropolis was undertaken in this study.

Method: The species are Aquaculture african catfish (*Clarias gariepinus*) and Giant sea catfish (*Arius gigas*) and their extracted oils labelled FO1 and FO2 respectively. Specific gravity (SG), Acid value (AV), saponification value (SV), peroxide value (PV), ester value (EV), antioxidant property, refractive index (RI), optical activity (OA) and GC-MS analysis were carried out on the oils.

Results: Results show values of 0.92/0.91 as SG for FO1/FO2, RI values for FO1/FO2 as 1.521/1.551; OA values of FO1/FO2 as -0.130/0; AV of FO1/FO2 as 2.95/ 1.53; SV for FO1/FO2 as 186.25/175.20 (mgKOH/g); PV of FO1/FO2 as 9.00/5.94 (mEq/kg) and EV of 183.30/173.67 (mgKOH/mg) for FO1/FO2. These physicochemical parameters fall within recommended ranges. The DPPH Antioxidant assay showed significant deviation from the standard at $P < 0.05$ ($P = 0.0003$). The GC-MS results showed that both FO1 and FO2 contain EPA, DHA, squalene, α-tocopherol and n-hexadecanoic acid. FO1 in addition contains linoleic acid, cholest-5-en-3-ol-(3β)-carbonochlorinate, a phenol and a dioxolane derivatives. FO2V in addition contains cholesterol, cholesterone and oleic acid.

Conclusion: The presence of dixolane and phenol derivatives in FO1 may be injurious to health.

Key words: antioxidant potentials, fish oils, gc-ms analysis, physicochemical parameters.

1. INTRODUCTION

Fish oils means oils intended for human consumption derived from fish and shell fish. Fish oils are primarily composed of glycerides of fatty acids and may contain other lipids and unsaponifiable constituents naturally present. Fish oils are considered as concentrated based on various processing which lead to higher levels of various polyunsaturated fatty acids (PUFA) [1]. The unique nature of fish oils depends on the variety of fatty acids they are composed of and their degree of unsaturation. Fish oil is an industrial product of great nutritional value due to its contents of long chain omega-3 polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), which are currently highly valued for their prophylactic and therapeutic properties in nutrition and health [2],[3]. DHA and EPA content of fish oil is an important quality parameter of this product. These fatty acids are related to different neuronal functions, and their absence is associated with diverse inflammatory processes and the precarious development of neurons in human patients. Likewise, their beneficial effects in cardiovascular diseases are recognized [4]. Functions of fish oil include raising the levels of high density lipoprotein (HDL) cholesterol, lowering of psychotic

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disorders in those who are at risk and supplementation with fish oil in high doses may reduce some symptoms of both schizophrenia and bipolar disorder [10]. Some studies indicate that fish oil in combination with diet triglycerides by about 15-30% and reduction of blood pressure in hypertensive patients [5],[6],[7]. Omega-3 fatty acids present in fish oil is reported to be essential for brain function [8]. Available evidence shows that people with certain mental disorders have lower omega-3 blood levels [9]. Fish oil supplements can reduce the chances of or exercise aid weight loss [11]. Benefits of fish oil have been shown in ameliorating inflammatory disorders, kidney related problems, diabetes, glaucoma, stomach ulcers, pancreatitis, asthma, dyslexia, eczema, obesity, weak bones, rheumatoid arthritis, cystic fibrosis, sickle cell disease and preventing weight loss caused by some cancer drugs [12]. In stressed and obese individuals, fish oil can reduce the production and gene expression of inflammatory molecules called cytokines [13]. Skin is the largest organ in the body, and it contains a lot of omega-3 fatty acids [14] and there are a number of skin disorders that may benefit from fish oil, including psoriasis and dermatitis [15]. Fish oil supplements during pregnancy and breast feeding may improve infant visual development and help reduce the risk of allergies [16]. Given that Omega-3 fatty acids make up a significant proportion of the brain, having enough of them may be important for preventing behavioural disorders in early life [17]. Fish oil supplements may improve perceived hyperactivity, in-attention, impulsiveness and aggression in children and this may be of remarkable benefit in childhood learning [18]. Studies show that fish oil may reduce asthma symptoms especially in early life [19]. In one review of nearly 100,000 people, a mother's fish or omega-3 intake was found to reduce the risk of asthma in children by 24-29% [20]. Furthermore, fish oil supplements in pregnant mothers may reduce the risk of allergies in infants [21]. Medical research suggests that these fatty acids might have a unique role to play in prevention of coronary artery disease (CAD) and the growth of different types of cancers [22]. The researcher discovered that the fish species picked for this research are sold mostly fresh either directly from the sea or from refrigerators (as medium of preservation) to the city dwellers. Besides, apart from oil present as integral part of the flesh of fishes, these fish species have localized fat pouch within their viscera that can easily be removed in sizeable quantities separate from other visceral content. Some nursing mothers in this locality obtain these fat, heat them into oil, and use them in nursing their infants. The fish vendors usually obtain their African catfish (*Clarias gariepinus*) from some people in the state who engage in cultivating these fish (aquaculture) as a source of self-employment or as a secondary source of income. Giant sea catfish (*Arius gigas*) are usually bought from fishermen fishing from river outlets leading to the coast of Atlantic Ocean known as the Bight of Biafra which Akwa Ibom State of Nigeria shares liturgical boundary with. There is some online information alerting on possible risk of eating farmed (aquaculture) fish with respect to methods of raising them [23]. Poor storage and preservation of fresh fish and fish products is a regular challenge in this environment especially because of non-steady power (electricity) supply to aid cold-room storage. One of the prominent component of the feeds used in raising these aquaculture fish is poultry by-product which on its own is adulterated by some poultry farmers with feed additives yet to be fully regulated in poultry farming. Akwa Ibom State is the largest producer of crude oil to the nation Nigeria, an outstanding member of OPEC. Once in a while there are incidences of crude oil spillage into the waterways in the course of oil exploration, the outcome of which can negatively affect aquatic life and the quality of fish and fish by-products in this environment. The research attempt to investigate the quality of oil that are obtained from fishes in this environment. Since the oil is an integral part of the fish, their quality directly reflects the quality of fish that are consumed in this locality. An average fish feed in the country contains these components in the product label: fish meal, soyabean meal, fine fish oil, meat and bone meal, poultry by-product meal, hydrolysed feather meal, wheat flour, wheat bran, rice bran, broken rice, corn fine grind, cassava meal, antioxidant, mould inhibitor, antimycotoxin, growth promoter, vitamins and minerals [24].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Equipment

Equipment used in this research were: Polarimeter, GC-Mass spectra detector (MSD), century electric oven, centrifuge, electric water bath, electronic weighing balance (Mettler Joledo, USA). The glass-wares including reflux apparatus were Pyrex products while the chemicals/reagents were of analytical grades and were obtained from JHD (England) BDH (UK) and Merck Chemical Ltd (England).

2.1.2 Biological Materials

Fish samples were obtained fresh from fish vendors at different locations in Uyo city and identified by Mr Aniebiet Inwang of the Department of fisheries and aquaculture, Ministry of Agriculture, Akwa Ibom State, Nigeria.

2.2 **Methods**

Aquaculture catfish (*Clarias gariepinus*), and giant sea catfish (*Arius gigas*) were used for this research work. Five of each fish species were sourced from different locations in Uyo. Fish abdomen was opened by cutting and the fats that envelope the viscera were removed and placed in beakers and labelled accordingly. Fats from each fish species were pooled together to give two sets of specimen. Each beaker was heated to 60-90^{0}C for approximately 30 minutes in the oven (Century, China). This process disrupts the vesicles holding the fat, thus releasing the leaking oil. The oil was centrifuged (4,000 rpm; 20 mins) to purify it using Uniscope laboratory centrifuge, Surgifriend Medicals, England. This is a modified 'wet pressing method' that involves direct removal of visceral fat, heat treatment and centrifugation. The clear yellowish oil on top was preserved in a refrigerator (Haier Thermocool, China) at $\leq 10^0$C while the sediments were discarded. Oil from *Clarias gariepinus* and *Arius gigas* were labelled FO1 and FO2 respectively.

2.2.1 Determination of specific gravity (SG)

This was carried out using 25 mL density bottle (W1) and the weight of each oil plus the bottle (W2) and the weight of the bottle plus equal volume of distilled water (W3) all at room temperature (25^{0}C). Specific gravity was calculated using equation 1.

$$SG = \frac{W2 - W1}{W3 - W1} \quad \text{----------} \quad 1$$

2.2.2 Determination of refractive index (RI)

The Abbe refractometer (Techmel, China) was used in this determination. Drops of each of the oil samples were transferred into the glass slide of the refractometer. N-hexane was circulated round the glass slide to keep its temperature uniform and the reading taken accordingly.

2.2.3 Determination of optical activity (OA)

This was determined using the polarimeter (Techmel, China). A blank sample (ethanol) was filled in the blank cell of the polarimeter holder and set to 'Zero'. A 1:1 solution of the oil in absolute ethanol was prepared and placed in the polarimeter cell and the optical activities of each oil taken.

2.2.4 Determination of acid value (AV)

Twenty-five millilitres (25 ml) of each of ethyl ether and absolute ethanol were measured into a conical flask. Five grams (5.0 g) of the oil was weighed and added. The mixture was shaken and 0.5 ml of phenolphthalein was added and agitated vigorously. The mixture was titrated with 0.1 M KOH until a pink colour that persisted for 15 seconds was observed. The acid value was calculated using equation 2 [25].

$$\text{Acid value} = (56.1V)/(W) \quad \text{----------} \quad 2$$

Where V is the difference in volume of KOH consumed in the titration, N is the molarity of KOH used and W is the mass of the oil sample. This was repeated twice and the average value taken. The same procedure was carried out on the other oil sample.

2.2.5 Determination of saponification value (SV)

Five grams (5.0 g) of the fish oil sample was weighed into a round bottom flask, 25 ml of 0.5 M ethanolic KOH was measured and added, and the mixture was boiled for about 30 minutes in a reflux condenser. The mixture was then titrated with 0.5 M HCl to neutrality. Blank titrations excluding the oil was performed. The titration was carried out thrice and the average volume of HCl used was determined. The saponification value for the oil sample was calculated using equation 3. This procedure was repeated for the other oil sample [25]

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$$SV = ((b\text{-}a) \times 28.05)/W \quad \text{----------} \quad 3$$

Where (b-a) is the difference in volume of hydrochloric acid consumed by the blank compared with the actual titrations while W is the weight of the oil sample used.

2.2.6 Determination of peroxide value (PV)

Three sets of five grams (5.0 g) of one of the oil sample was weighed into a 250 ml conical flask. Thirty millilitres (30 ml) of glacial acetic acid and chloroform (3:2) was measured and added to each of the oil samples and swirled to dissolve. One millilitre (1 ml) of saturated KI solution was added to each of the solutions. The solutions were kept for one minute in a dark cupboard with occasional shaking. Thirty millilitres (30 ml) of distilled water was measured and added to each. The liberated iodine was titrated with 0.01 N sodium thiosulphate solution until the blue colour disappeared, using starch solution as indicator added towards the endpoint. The titration was carried out on the three samples and the average volume of sodium thiosulphate used was determined. The saponification value of each of the fish oil sample was calculated using equation 4 [26]. This procedure was repeated for the other oil sample.

$$PV = \frac{V \times N \times 1{,}000}{W} \quad \text{----------} \quad 4$$

Where V is the volume of the sodium thiosulphate, N is the normality used for the titre and W is the weight of the fish oil sample.

2.2.7 DPPH free radical scavenging antioxidant assay

The 2,2-Diphenyl-1-Picryl (DPPH) free radical scavenging capacity of the fish oils were evaluated according to the method designed in this study. Various concentrations (50-500 µg/mL) of the oil samples were added to 2 ml of prepared n-hexane DPPH solution (0.1 nm). The mixture was shaken and left to stand at room temperature in a dark cupboard for 30 minutes. The absorbance was measured at 517 nm against a blank (the blank contained all reagents except the test sample). The assays were carried out in triplicates. The concentrations of the sample that gave 50% inhibition of DPPH ($1C_{50}$) were obtained from a plot of percentage inhibition (%I) versus concentration of the sample in µg/ml. The percentage inhibition of DPPH (%I) was calculated using equation 4.

$$(\%I) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

A_{blank} is the absorbance solution and A_{sample} is the absorbance of the test sample. Vitamin C was used as positive control.

2.2.8 Gas Chromatography-Mass Spectroscopy (GC-MS)

GC-Mass spectra detector, MSD; Model 5977A, Agilet Technologies, California, USA, was used for this analysis. This is gas chromatography coupled to an ion trap mass spectrometer using ionization energy of 70Ev and He as the carrier gas. Maximal column temperature of 240^{O}C was employed and the samples were analysed in the full scan mode. The obtained spectra and fragmentation were recorded.

2.3 Statistical Analysis

Data obtained was analysed with GraphPad prism Version 8.0.2 for windows. The same software was used in the plotting of the graphs. Data analysis was carried out using ANOVA and standard deviation.

3. RESULTS

3.1 Physicochemical properties of the fish oil samples

Table 1: Physicochemical properties of the fish oil samples

Parameters	**Experimental Value**		**Standard Value**	
	FO1	FO2	ASTM, 1952[30]	FAO, 2017[31]
Appearance/Colour	Light Yellow	Deep Yellow		
Specific gravity	0.92	0.91	0.907-0.915	
Refractive Index	1.521	1.511	1.400-1.473	
Optical Activity	-0.130	0		
Acid Value (mgKOH/g)	2.95	1.53		≤ 3

Saponification Value (mgKOH/g)	186.25	175.20	175-201	
Peroxide value (mEq/kg)	9.00	5.94	< 10	≤ 5
Ester value (mgKOH/g)	183.30	173.67		

3.2 Antioxidant (DPPH) free radical scavenging

Table 2: DPPH free radical scavenging activities of fish oils

Conc. (µg/ml)	Vitamin C (%1)	FO1 (%1)	FO2 (%1)
50	74.42	72.38	70.00
100	78.86	72.94	71.18
150	83.41	73.49	71.27
200	85.26	74.00	72.00
250	89.89	74.24	72.01
500	93.42	75.00	74.14

%1: Percentage inhibition

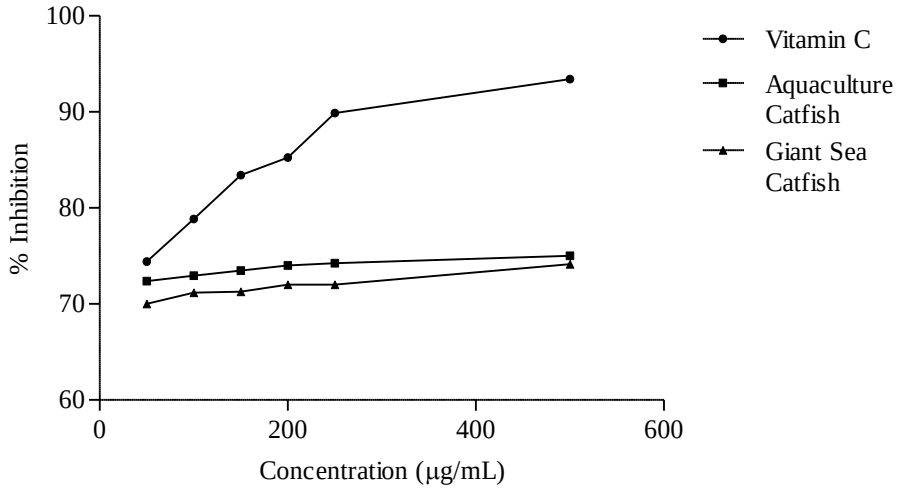
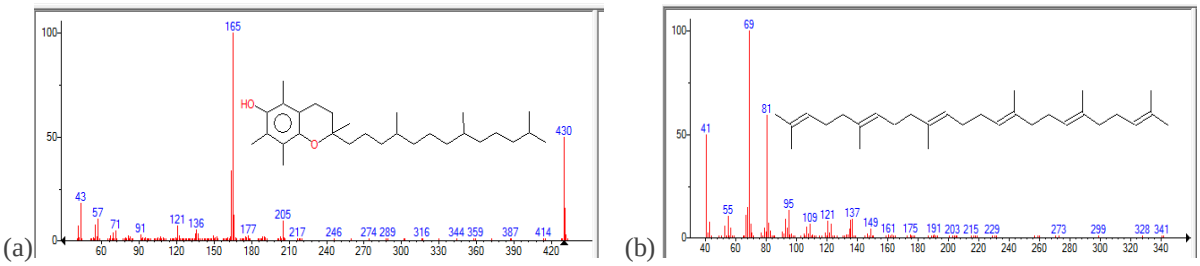


Figure 1: Graph of % DPPH inhibition versus concentration for antioxidant activities of FO1 and FO2 compared to standard

3.3 Result of GC-MS spectral study

(a) Compounds identified from (FO1) using GC-MS are shown on figure 2 and 3.
(b) Compounds identified from (FO2) using GC-MS are shown on figure 2 and 4.



(c)

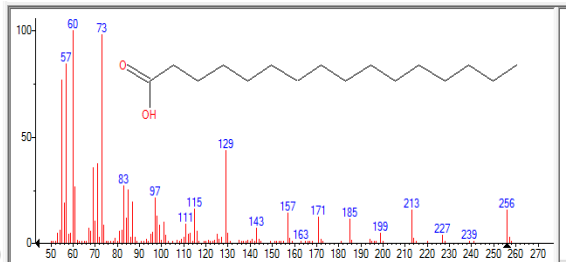
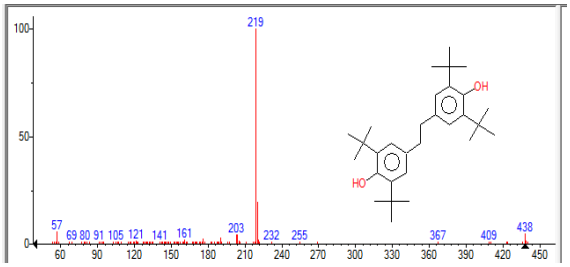
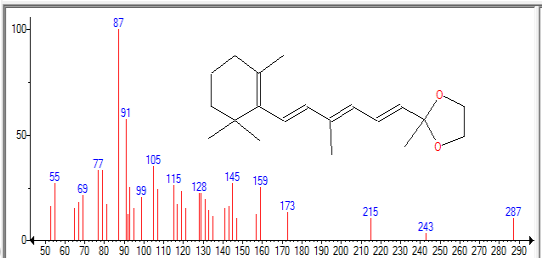


Figure 2: Mass spectra/structures of compounds identified in both FO1 and FO2: (a): dl-α-tocopherol, (b): Squalene, (c): n-hexadecanoic acid.

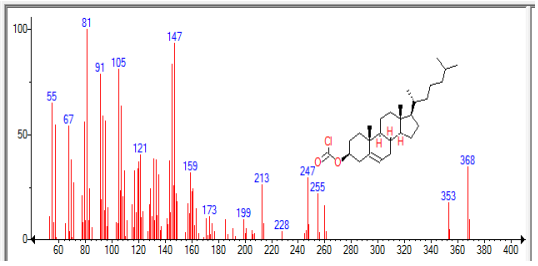
(a1)



(b1)



(c1)



(d1)

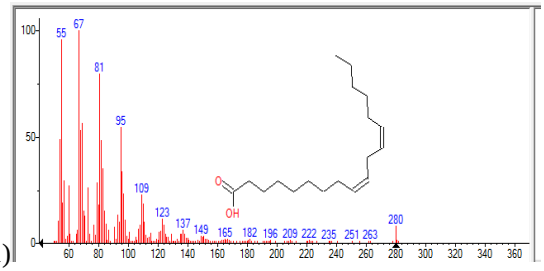
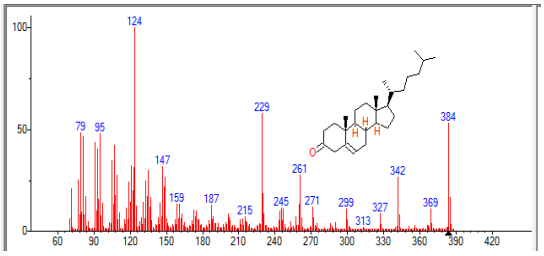
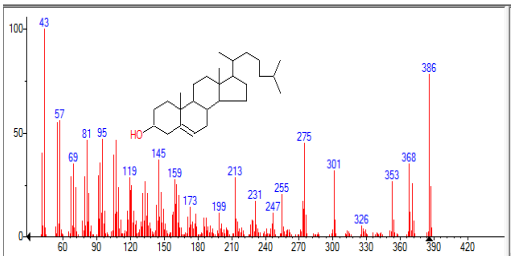


Figure 3: Mass spectra/structures of compounds identified in FO1: (a1): 4,4/-Ethylene-2,6-ditertbutyl phenol, (b1): 2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatriene]-1,3-dioxolane, (c1): Cholest-5-en-3-ol-(3β)-carbonochlorinate (d1): Linoleic acid

(a2)



(b2)



(c2)

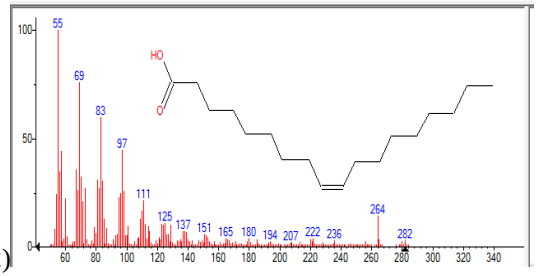


Figure 4: Mass spectra/structures of compounds identified in FO2: (a2): Cholesterone, (b2): Cholesterol, (c2): Oleic acid

4. DISCUSSION

The values of specific gravities for the two oils fall within accepted standard (0.907-0.915). The deviation of their refractive indices 1.521/1.511 for FO1/FO2 from the standard (1.400-1.473) is statistically non-significant at $P > 0.05$. Optical activities of FO1/FO2 (-0.130/0) show that FO1 has components that leads to a net laevorotatory state while FO2 might be a racemic mixture. Acid value is the number of milligram of KOH required to neutralize the free fatty acids present in most oil and can be used to reject low grade, rancid oils and fats which contain larger quantities of free acids [29]. Acid value of FO1/FO2 (2.95/1.53) fall within the standard value (≤3). Saponification value is the number of mg of KOH required to neutralize the fatty acids obtained by complete hydrolysis 1.0 g of the material (together with the free fatty acids already present in the oil). It is therefore a measure of total acids present and gives indication of purity [29]. SV for FO1/FO2 (186.25/175.20 mgKOH/g) falls within standard (175-201 mgKOH/g). Peroxide value (PV) is the number which expresses in milliequivalents of active oxygen, the amount of peroxide contained in 1,000 g of the substance. High peroxide values are indicative of poor quality oils [29]. PV of FO1/FO2 (9.00/5.94 mEq/kg) falls within standard (<10 mEq/kg). Ester value is the number of mg of KOH required to neutralize the fatty acids obtained solely by hydrolysis of the glycerides contained in 1.0 g of the substance. It is the difference between SP and AC [29]. EV for FO1/FO2 183.30/173.67 mgKOH/mg). This values are close to their saponification values and showed that the fish oils had low free fatty acids but exists mostly as glycerides esters. These physicochemical parameters fall within recommended ranges. Besides, these physicochemical parameters are dependent on geographical locations, seasons and purpose for which the oil will be used [30]. In this research, their values are within stated standards. The DPPH Antioxidant assay showed significant lower deviation from the standard at $P < 0.05$ ($P = 0.0003$) indicating that the oils are poor antioxidants compared to vitamin C. The GC-MS results showed that both FO1 and FO2 contain EPA, DHA, squalene, α-tocopherol and n-hexadecanoic acid. FO1 in addition contains Linoleic acid, Cholest-5-en-3-ol-(3β)-carbonochlorinate, a phenol and a dioxolane derivatives. FO2V in addition contains cholesterol, cholesterone and oleic acid. The presence of dixolane and phenol derivatives in FO1 need to be further evaluated for their source and their safety when consumed.

5. CONCLUSION

The usefulness of fish oil is optimized when it is available in a pure unadulterated form either in extracted or encapsulated form or eaten directly as fish meal. The raising and processing of such fishes and their oils should be done in such a way that the health of the consumers are not jeopardized. The phenolic and dioxolane derivatives found in aquaculture African cat fish might have had entrance through fish feeds or ponds. These compounds need further evaluation to determine its safety and stop its entrance into the fish body and fish oil.

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Conflict of Interest

There is no conflict of interest.

Contribution of the Authors:

This work was carried out in collaboration with all authors. Author VUA designed the study and performed the experimental procedures. ASE, SJO and RAU wrote the first draft of the manuscript. NJO and MEA reviewed literature and assist in fish collection and preparation. All authors read and approved the final manuscript.

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