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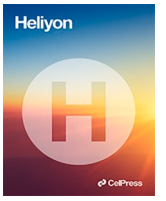

Research article

Leaves of *Allium cepa* and *Xylopia aethiopica* fruits as potent sources of natural antioxidant for stabilization of viscera FISH oil during accelerated storage

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ABSTRACT

The present study investigated the antioxidant activities of methanolic extracts of two local plants and their capacities to preserve the quality of viscera fish oil during storage. The total phenolic, total flavonoids and tannins contents were analyzed by colorimetry, the preliminary antioxidant tests done and identification of phenolic compounds by HPLC-DAD (Diode Array Detector) and ESI-MS. After these evaluation, the plant extracts were added in viscera fish oil at concentration 200–1000 ppm. The oxidative stability of these fish oil samples was evaluated by measuring their qualities indices during 16 days at 70 °C. Here, oils were collected at the day 0, 4, 8, 12 and 16. The total phenolic, flavonoids and tannins contents in *Xylopia aethiopica* fruits were 15.62 g Eq acid galic/100g of dry extract, 10.85 mg Eq quercetin/100g of dry extract and 0.79 mg Eq cathechin/100 mg of dry extract. Those of *Allium cepa* leaves were 6.85 g Eq acid galic/100g of dry extract, 1.50 mg Eq quercetin/100g of dry extract and 0.11 mg Eq cathechin/100 mg of dry extract. All the methanolic extracts exhibited antioxidant activity. The results recorded after FRAP assay revealed the low IC50 (12.87 mg/mL) in *Allium cepa* extract compared to that of *Xylopia aethiopica* extract (44.90 mg/mL). These local plants contain many classes of phenolic compounds and they can be used in oil and fat industries as alternative of synthetic antioxidants.

1. Introduction

For improving human nutrition and food security, the sector of fishing and aquaculture is crucial. The demand of fish by the consumer's increases continuously. Fish can be considered as a treasure store of nutrients. Due to their good sources of nutritional component, many marine foods like fish recently have attracted more attention from consumers [1]. Fish protein contains all the essential amino acid for human health and their inclusion in mixed food can ameliorated their protein quality. Many fatty fish are sources of unsaturated fatty acids like long chain omega-3 fatty acids which contribute to the visual and cognitive human development, especially during the first three month of child's life. The production of fish oil can be obtained from their by-products, including

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heads, skins, skeletons and viscera [2]. Viscera is the highest quantity of fish processing by-products that occupies 12–18 % of the whole fish body weight. As the highest portion of fish by-products, fish viscera have a great interest to explore for having added value products. Furthermore, fish viscera is a valuable source of several compounds which are essential for food industries. The composition of fish viscera to muscle and head has higher lipid and cholesterol, more saturated fatty acid but has higher ω-3 fatty acids. Fat is the highest component of fish viscera generally after moisture content. Depending on the fish species, fish viscera oils contain ω-3 fatty acids that are potentially used as foods supplements [3]. Eicosapentaenoic acid (EPA) and docosahexaenoic acids (DHA) are the main polyunsaturated fatty acid present in fish viscera oil. Both fatty acids play many beneficial roles in human health with the benefits are the great interest of the food and pharmaceutical industries [4]. In addition to ω-3 fatty acids, viscera fish oil contains vitamins A and E which are natural antioxidants [5].

In the world, cardiovascular diseases and coronary heart diseases have been identified as causes of dead. Consumption of fish oil is therefore being encouraged due to their high unsaturated fatty acids. Eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), an important polyunsaturated fatty acid present in marine and freshwater fish oil has a preventive effect on neurodevelopment in infants [6]. EPA is a precursor of prostaglandins, leucotriens and thromboxanes. DHA is a component of phospholipids membranes of brain and retinal cells, it is essential for human health [7].

Fish oil remains the most perishable product after their extraction. Their alteration increase rapidly during storage and processing. The high unsaturated fatty acids in fish oils make them more susceptible to oxidation and its shelf life is limited by enzymatic and microbial activity [8]. Oxidation of polyunsaturated fatty acid is catalysed by heat, light and enzymes, and leads to the formation of peroxides, aldehydes, ketones and free radicals which are dangerous for consumers [8]. Lipid oxidation lead to an unpleasant flavour, aroma and taste making oil unsuitable for human consumption. To solve the deterioration reactions, synthetic antioxidants such as butyl hydroxyl toluene (BHT), butyl hydroxyl anisole (BHA) have been used as food additives. However, many report reveals their implications in many diseases such as cancer, cardiovascular diseases and their use becomes restricted in many countries [9]. BHA and BHT in others hand are quite volatile and easily decomposed at high temperature [10]. In the field of lipid oxidation, as a result, natural antioxidant such as plant extract, essential oil received a lot of attention. Bouba et al. [11] has reported that the majority of natural antioxidant are phenolic compound or polyphenols. Their activities are based on their structure, hydrogen donating potential, ability to chelate metal ions and their synergic action. Many studies present the use of natural antioxidant to prevent lipid oxidation [12–16], but only few are authorized. It is so important to look some others one.

Allium cepa has been used in traditional medicine for the treatment of diabetes. It has been used as a diuretic to reduce swelling. *Allium cepa* also help to reduce arteriosclerosis by lowing blood cholesterol level and prevent the formation of blood clots [17]. It is use to prevent bacterial and fungal infections [17]. *Xylopia aethiopica* traditionally has been used to treat femal infertility, hemorrhoids, bronchitis, oedema, syphilis, diabetes, dysentery and stomach aches [18,19]. Fleischer [20] reported that the fruit of *Xylopia aethiopica* has antipyretic and anti-inflammatory properties. It exhibited antibacterial and antifungal activities [21]. *Allium cepa* and *Xylopia aethiopica* contains phenolic compounds [22]. These compounds have antioxidant activities and can be used to prevent oil oxidation during storage. No study has been reported on the effect of these plant extract on the prevention of lipid oxidation of fish oil. The main objective of this study is to evaluate the effect of the extract of *Allium cepa* leaves and *Xylopia aethiopica* fruits on lipid oxidation of crude viscera fish oil during accelerated storage. The viscera fish oil was used in this study because in the Far North Cameroon, these oils are commercialized by the fisher man. They are the sources of unsaturated fatty acid.

2. Material and methods

2.1. Material

In this study, all chemicals and solvents used were of analytical grade and were procured from Sigma-Aldrich, st. Louis. Standard antioxidants (BHT, 2,2-diphenyl-1-pacrylhydrazyl (DPPH), quercetin), methanol, chloroform, glacial acetic acid, potassium iodide, sodium thiosulphate, potassium hydroxide, sodium acetate, vanillin, trichloroacetatic acid (TCA), ferric trichloride (FeCl3), folin-ciocalteu, reagent were procured from Carlo Erba reagents.

Crude viscera fish oil was purchased from the fisherman market at Maga in Far-North, Cameroon and then stored at 4 °C in refrigerator until used. This oil was obtained traditionally by mixing the viscera with sufficient quantity of drinkable water. The reason of their use is due to their avaibility in Maga local market and their utilization. Fresh leaves of *Allium cepa* were collected in Maroua, Diamare division, Far-North region of Cameroon on February 2022 from famers. The fruits of *Xylopia aethiopica* were purchased in Maroua local market in February 2022. This area is located between latitude 10° and 13° North between longitude 13° and 16° East. After collection, the plants were transported in the Biochemistry laboratory, University of Maroua, Maroua Cameroon.

2.2. Methods

The cut leaves of *Allium cepa* and the fruit of *Xylopia aethiopica* were washed thoroughly with tap water to remove any wastes and dust particules. The leaves and fruits were dried in electric air dried oven (Memmert UN 30, Zindorf) at 50 °C for 48h. The dried plants were powdered using a blender food (Panasonic, Kyoto, Japan) to facilitate the penetration of the extraction solvent into the cell. The powder sample obtained can pass through a 1 mm sieve. The plant powders were kept in the desiccators for further use. The plant extract was prepared following the method reported by Womeni et al. [13]. For 48 h at room temperature, about 100 g of obtained powder was extracted into 800 mL of methanol. During extraction, the mixture was regularly subjected to shaking. The extract was filtered with a watman N° 1 filter paper and residues again extracted with 400 mL of methanol to ensure the maximum extraction of

phenol compounds. The filtrate was concentrated using vacuum evaporator at 40 °C under reduced pressure for removal of solvent. After that, the dried extract obtained was stored at 4 °C prior to further analysis.

2.3. Quantitative phytochemical analysis

2.3.1. Total phenolic content

The Folin ciocalteu colorimetric method described by Gao et al. [23] was used to determine the total phenolic content of the plant extract. Briefly, 20 μL of plant extract were mixed in test tube with 0.2 mL of Folin-ciocalteu reagent and 2 mL of distilled water and incubated at room temperature for 3 min. After that, 1 mL of 20 % sodium carbonate was added to the mixture and reincubated for 2h at room temperature. The absorbance was measured at 765 nm using a quartz cuvet as standard, gallic acid was used and the total phenols content was expressed as mg of gallic acid equivalent per g dry weight.

2.3.2. Total flavonoid content

The aluminum chloride colorimetric method described by Pallab et al. [24] was used to determine the total flavonoid content of the different crude extract. Approximately 0.1 g of plant extract was homogenized with 10 mL of methanol. 1 mL of aluminum chloride reagent was added to 0.1 mL of the mixture diluted to a tenth. After homogenization, two drops of acetic acid were added. The mixture was again homogenized and absorbance at 430 nm was read using spectrophotometer (Varian 220FS Spectr AA, Les Ulis, France). The total flavonoid content was calculated from a calibration curve of quercetin standard solution and the result was expressed as g of quercetin equivalents per 100g of plant extract (g QUE/100 g of dried extract).

2.3.3. Total tannin content

Total tannin content of the extract was estimated using the method described by Naima et al. [25]. Essentially, 0.1 g of plant extract was homogenized with 10 mL of methanol. 2 mL of reactive reagent was added to 0.2 mL of mixture diluted to a tenth. The absorbance was read at 500 nm after the mixture was incubated at 30 °C for 5 min the total tannin was calculated from the calibration curve of catechin standard solution and expressed in grams of catechin equivalents per 100 g of plant extract (g CAE/100 g dry extract).

2.3.4. High-Performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS) of the extract

HPLC paired with ESI-MS was used to analyze the composition of phenolic compound in the extracts. For that, 50 mg of dried material was dissolved in 1200 μL of methanol containing 1 % of acetic acid, followed by sonication for 15 min (Brasson, 2200, USA). For three times, the procedure was repeated and the combined extracts were filtered through an injection flask (Mini uniprep whatman 0.45 μm) for HPLC and injected into an HPLC-UV-visible-DAD-ESI-MS system. An autosampler Model Surveyor, Thermo Scientific), an SCM 1000 degasification system (Thermo Scientific), a diode array UV–visible detector (DAD, UV6000LP, Thermo Scientific) and a 1100 series binary pump (Agilent Technologies) composed the analytic device. The mass spectrometer (MS) was ion trap (LCQ Deca, Thermo Scientific) equipped with and ESI source. A volume of 2 μL of sample was injected into a Purospher STAR Hibar HR RP18 column (150 mmx2.1 mm, 3 μm, thermostated at 30 °C, Supelco). A solvent A (aqueous solution of 0.1 % formic acid) and solvent B (acetonitrile containing 0.1 % formic acid, v/v) constituted the mobile phase. The following linear gradient elution was applied at a constant flow rate of 0.2 mL/min: initial, 3 % B; 0–3 min, 7 % B, linear; 3–21 min, 13 % B, linear; 21–27min, 13 % B, linear; 27–40 min, 30 % B, linear; 40 (51 min, 50 %, linear; 51–53 min, 90 % B, linear; 53–56 min, 90 % B, linear; 56–58 min, 3 % B, linear; followed washing and reconditioning of the column. The UV–visible detection was performed in the 240–600 nm range. The ESI source was used in negative mode. The MS detection was carried out with the following parameter: MS spectra were acquired in full-scan negative ionization mode in the m/z 50–2000 range to obtain the signals corresponding to the deprotonated (M − H) molecular ions. The method included the MS/MS-dependent scan mode which was used to obtain the product ion spectrum of the main molecular ions detected on the chromatogram in the full-scan mode. The collision energy was optimized at 35 % (arbitrary units) to clearly observe the production of both parent and main daughter ions. Data were collected and processed by XCalibur Software (version 1.2, Thermo Finigan).

By comparison with available standards, the retention times, UV–Vis spectra, full MS spectra and MS/MS spectra were used for complete identification. When the standard was not available, the criteria were used for partial identification only. Quantifications were carried out by integration of the peaks on UV–Vis chromatograms at 280 nm for flavavols, 320 nm for hydrocinnamic acids, 350 nm for flavanols and 520 nm for anthocyanins.

(−)-Epicatechin, 5-caffeoylquinic acid and procyanidin dimer B2 were quantified according to their own calibration curves, whereas other compounds were quantified “as equivalents” according to a reference compound belonging to the same polyphenol class and presenting a comparable UV–Vis spectrum. Thus procyanidin oligomers were quantified in epicatechin equivalents, flavonols in hyperoside equivalents and anthocyanins in ideain equivalents.

2.3.5. Preliminary antioxidant tests

2.3.5.1. DPPH radical scavenging assay. The antiradical activity of the extracts were evaluated with colorimetric method using the radical 2,2diphenyl 1- picrylhydrazyl (DPPH), as decribed by Braca et al. [26]. To 0.5 mL of different concentrations of samples (125, 250, 500, 1000, and 2000 μg/mL), 5.5 mL of 0.002 % alcoholic solution of DPPH was added and standard solutions sparely, in order to have final concentration of products of 125–200 μg/mL. As positive control, a synthetic antioxidant, Butyl aldehydroxytoluene (BHT)

Table 1
Total phenolic, flavonols and tannins content of *Xylopia aethiopica* fruits and *Allium cepa* leaves extracts.

Extracts	Total phenolic content (g GAE/100g dry extract)	Flavonoïdes (mg QUE/100g dry extract)	Tannins (mg CAE/100g dry extract)
Xylopia aethiopica **fruits**	15.62 ± 0.20^{a}	10.85 ± 0.00^{a}	0.79 ± 0.01^{a}
Allium cepa **leaves**	6.85 ± 0.13^{b}	1.50 ± 0.17^{b}	0.11 ± 0.02^{b}

Values with different letters in the same column are significantly different at the probability threshold $p< 0.05$; Mean ± Standard deviation; $n = 3$.

was used. For 30 min, at room temperature and in the dark, the mixtures were kept. After that, the absorbance of the mixture, control and blank were measured at 517 nm in comparison with methanol. The ability of the sample to scavenge DPPH radical was determined using the following formula:

$$AA\% = [(\text{Abs control} - \text{Abs sample}) \text{ X100} / \text{Abs control}]$$

2.3.5.2. Ferric reducing antioxidant power. The FRAP test is a typical SET-based method measuring the reduction of the complex of ferric ions (Fe^{3+})-ligand to the intensely blue ferrous complex (Fe^{2+}) by means of antioxydants in acid environments. The ferric reducing power of plant extracts were measured using the method described by Oyaizu [27]. Briefly 0.5 mL of plant extract at different concentrations (125, 250, 500, 1000, and 2000 μg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL (1 %) of potassium ferricyanide $K_3Fe(CN)_6$, well shaking and incubated at 50 °C for 30 min after incubation, the reaction was stopped by adding 1 mL of 10 % (w/v) trichloroacetic acid, followed by centrifugation at 500*g* for 10 min. Finally, 1.5 mL of the upper layer was mixed with 1.5 mL of distilled water and 1.1 mL of feCl3(0.1 %) and absorbance was measured at 7000 nm on spectrophotometer. The sample concentration providing 0.5 of absorbance IC50) was calculated by plotting absorbance against the corresponding sample concentration. A sample blank containing all the reagents but no extract was prepared in the same condition. BHT was used as a reference compound. The reducing antioxidant power was calculated from the calibration curve of ascorbic acid (0–125 μg/mL) standard solution and expressed in mg of ascorbic acid equivalents per 100 g of plant extract (mg AsAE/100 g of dry extract) by using the following formula:

$$\text{FRAP (mgASAE / 100 g dry extract)} = [\text{Abs x Fd x V x 100 / (Tp x a)}]$$

were Fd = dilution factor;

V = total extraction; Tp = test portion; a = slope of the calibration curve.

2.4. Effect of plant extract on viscera fish oil oxidation

2.4.1. Samples preparation

A crude concentrated methanolic extract of *Allium cepa* leaves and *Xylopia aethiopica* fruits were dissolved in a few volume of methanol and separely added to 100 g of preheated viscera fish oil (at 70 °C for 96h) at concentration 200, 400, 600, 800 and 1000 ppm. Synthetic antioxidant (BHT) was used at the legal limit of 200 ppm [28] to compare the efficacy of natural antioxidants. A viscera fish oil sample, free from additives and prepared under the same conditions was used as control. The oxidation rates of all samples of viscera fish oil were followed in an oven at 70 °C for 16 days. Samples were withdrawn (20 g approximately) periodically every 0, 4, 8, 12 and 16 days for analysis. Immediately after storage period, oil samples were analyzed.

2.4.2. Measurements of viscera fish oil oxidation

The free fatty acids content (% oleic acid) and peroxide value of viscera fish oils were determined according to the AFNOR [29] procedures. Concerning FFA, the oil sample (1g) was dissolve in 100 ml of ethanol and some drop of phenolphthalein were added as an indicator and swirled vigorously. The mixture was then titrated with potassium hydroxide (0.1 N). The FFA was expressed as % oleic acid. For PV, the oil sample (1.0 g) was treated with 25 mL of organic solvent mixture (chloroform: acetic mixture, 2:3). The mixture was shaken vigorously, followed by addition of 1 mL of saturated potassium iodide solution. The mixture was kept in the dark for 5 min and 75 mL of distilled water were added and the mixture was shaken. To the mixture, 0.5 mL of starch solution (1 %, w/v) was added as an indicator. The peroxide value was determined by titrating the iodine liberated from potassium iodide with standardised 0.01 N sodium thiosulfate solution. The PV was expressed as milliequivalents of free iodine per kg of lipid. The procedures described by O'keefe and Pike [30] and Drapper and Hadley [31] were respectively used to evaluate the iodine and thiobarbituric acid values in oils. The *P*-anisidine value was determined according to a modification of AOCS's official method Cd 18–90 (AOCS, 1998). The Total oxidation (TOTOX) values of oil samples were determined based on the obtained peroxide and *p*-Anisidine values using the equation TOTOX value = PV + AV, according to Shahidi and Wanasundara [32].

2.5. Statistical analysis

The results reported are the averages of three replications. Data were analyzed by one-way analysis of variance (ANOVA) using Statistical Package of Social Science (SPSS 16.0 version). Comparison was made between species and treatments. Significance level was set at $P < 0.05$.

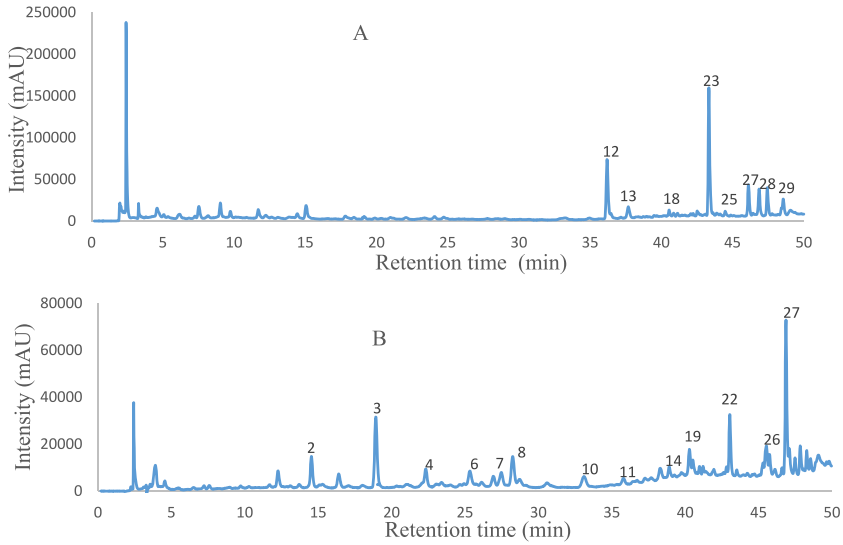

Fig. 1. Reversed-UV visible HPLC chromatogram of methanolic extracts of *Allium cepa* leaves (A**)** and *Xylopia aethiopica* fruits (B): (peak 2) isomer 1 of 5-cafeoylquinic acid (hydroxycinnamic acid); (peak 3) *p*-coumaroylquinic acid isomer 1 (hydroxycinnamic acid); (peak 4) cafeoylquinic acid isomer 2 (hydroxycinnamic acid); (peak 6) procyanidin dimer B2 (flavan-3-ol); (peak 7) (−)-epicatechin (flavan-3-ol); (peak 8) *p*-coumaroylquinic acid isomer 2 (hydroxycinnamic); (peak 10) procyanidin trimer (flavan-3-ol); (peak 11) procyanidin tetramer (flavan-3-ol); (peak 12) dihexoside quercétin (flavonol); (peak 13) dihexoside isorhamnetin (flavonol glycoside); (peak 17) quercetin-3-*O*-glucuronide (flavonol glycoside); (peak 18) quercetin-*O*-hexoside (flavonol glycoside); (peak 23) quercétin-*O*-hexoside (flavonol glycoside); (peak 25) quercétin-3-*O*-glucoside (flavonol glycoside); (peak 29) quercetin (flavonol).

3. Results and discussion

3.1. Total phenolic, flavonoids and tannins contents of plant extracts

The contents of total phenolic, flavonoids and tannins of methanol extract of leaves of *Allium cepa* and *Xylopia aethiopica* fruits are presented in Table 1.

Phenolic compounds are a groups of secondary metabolites with highly effective free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and antiflammatory action [33]. As shown in Table 1, these plants extracts were characterized by presence of considerable amount of phenolic compound. The highest content (15.62 g Eq acid galic/100g of dry weight) was found in *Xylopia aethiopica*, while the lower value (6.85 g Eq acid galic/100g of dry weight) was found in *Allium cepa* leaves. The variation noted may be due to the species, the part of plant tested and the quality of sample collected. The total phenolic content noted in the present study were higher than 5.23 g Eq acid galic/100 g of dry weight, value obtained by Sajjad et al. [14] in Pakistan with leaf extract of *Eucalyptus citriodora.* These may be due to the specie. Concerning *Allium cepa*, their value is higher than 0.479 Eq acid galic/100 g of dry weight and 0.229 g Eq acid galic/100 g of dry weight respectively obtained by Bibi et al. [34] with red flame *Allium cepa* species cultivated in 3 localities in Pakistan. Wani and Basin [35] recorded 7.18 g Eq acid galic/100 g of dry weight with methanol extract of *Allium sativum*. Regarding *Xylopia aethiopica*, their total phenolic content found was lower than 42.8 g Eq acid galic/100 g of dry weight, value noted by Lawal et al. [36] with methanol extract of the same species. Variation may be link to environmental conditions and extraction conditions.

Flavonoids are a group of phenolic compounds with beneficial abilities ranging from their ability to scavenge a wide range of oxygen, peroxyl radicals and hydrochlorious acid to their ability to chelate ions by decreasing the metal ions pro oxidant capacity [32]. The total flavonoids content (TFC) of the two plants used in this study are ranged from 1.50 mg Eq quercitine/100 g dry extract (methanol extract of *Allium cepa* leaves) to 10.85 mg Eq quercitine/100 g dry extract (methanol extract of *Xylopia aethiopica* seeds) (Table 1). The seeds of *Xylopia aethiopica* was the richest in flavonoids and this could be due to their strong coloration. John and Takayuki [37] shown that the color of certain fruits and vegetables is attributed to flavonoids and intensity of this coloration is strongly correlated with the flavonoids content. Results in this study on the leaves onion flavonoid content were lower than those noted by Mardani et al. [38] on onion layer of azarshahr red onions in Iran (1.92 mgEq quercitine/100 g dry extract) and still very lowest than 22.7 mg Eq quercitine/100 g dry extract, value noted by Santos et al. [39] on red onion methanolic extract. Dziri et al. [40] mentioned that red onion is rich in flavonoids due to their coloration. Bibi et al. [34] noted the higher value of TFC on leaves extract of onion varieties planted under diverse environmental conditions in Pakistan. Variation observed could be link to various environmental factors and genetic variation among the plant species. Strong correlation was noted between altitude and TFC, indicated the impact of elevation on the synthesis of secondary metabolites (Bibi et al., 2022). TFC of methanolic *Xylopia aethiopica* seeds in this study is lower than 428.25 mg Eq quercitine/100 g dry extract noted by Oso and Oladiji [41] in the aqueous extract of the same fruit. Difference may be link to extraction solvent and environmental conditions.

Table 2
DPPH scavenging activity of *Xylopia aethiopica* fruits and *Alium cepa* leaves extracts.

Extracts	Extract concentrations in µg/mL				
	25	50	100	150	200
Xylopia aethiopica fruits	30.00 ± 0.00^{e}	32.5 ± 0.71^{d}	38 ± 0.00^{c}	45 ± 0.00^{b}	49 ± 0.00^{a}
Allium cepa leaves	28.00 ± 0.00^{c}	28.00 ± 0.00^{c}	28.5 ± 0.71^{b}	31.00 ± 0.00^{a}	31.00 ± 0.00^{a}
BHT	36.11 ± 0.41^{e}	40.20 ± 0.33^{d}	64.35 ± 0.16^{c}	76.07 ± 1.12^{b}	80.50 ± 0.16^{a}

Values with different letters in the same line are significantly different at the probability threshold $p< 0.05$; Mean ± Standard deviation; $n = 3$.

Table 1 also reveals the total tannins content of two local plants consumed in Cameroon. Tannins possess astringent properties to quicken the healing of wounds [42]. The total tannins content of kimba (0.79 mg cathechine/100g dry extract) was significantly higher ($P < 0.05$) than those of onion leaves (0.11 mg cathechine/100 g dry extract). The observed differences may be due to the specie, the harvesting time and climatic conditions of the growing area. The tannins content of *Xylopia aethiopica* noted in this study is higher than that noted by Obiloma et al. [43] in the same part of plant in Nigeria. Concerning the leaves of onion, the tannins content in the present study is lower when compared to the tannins content in various onion varieties previously reported by Bouhenni et al. [44] in Algeria. The variation in values might be due to the different cultivation conditions.

3.2. Phenolic antioxidant detected by HPLC on the two plant extracts

Identification of phenolic compounds in the two plants extracts were done by the means of liquid chromatography (HPLC) coupled to mass spectrometry (MS) using the electrospray ionization interface (ESI). HPLC with diode array detector (DAD) was carried out for quantification of phenolic compound in these extracts.

As shown in Fig. 1(A), for *Allium cepa*, eight phenolic compounds were detected and five were identified as a flavanols glycoside and one flavanol. The peaks 12, 13, 18, 23, 25, and 29 were found as matching well with Quercitin di-hexoside (RT: 36.15 min), Isorhamnetin-di-hexoside (RT: 37.6 min), Quercitin-*o*-hexoside (RT: 40.55min and RT: 43.3 min), Quercitin-3-o-glucoside (RT: 44.47 min) and Quercitin (RT: 48.55 min) respectively. Another's analysis were needed to identified the three unidentified phenolic compound present in *Allium cepa* leaves. Among the flavonoids, Quercetin-*o*-hexoside, Quercetin di-hexoside and Quercetin were found with higher content and Quercetin-*o*-hexoside was the important phenolic compound detected in *Allium cepa* leaves (1227 mg EH/Kg of dry powder). All the compound detected are well known as strong antioxidant. In the bulb of *Allium cepa*, the abundant phenolic compound identified was Quercetin −3,4′-diglucoside [45], while in the flower, Isorhamnetin-3-o-glucoside was found to be the important phenolic compound. The variation noted may be explain by the plant part, the growing condition and harvest season.

The HPLC-DAD of analysis of *Xylopia aethiopica* fruits methanol extract is presented in Fig. 1(B). Thirteen phenolic compounds were detected and nine were identified: four hydroxycinnamic acid, 4 flavanols, and one flavanol glycoside. The peaks 2, 3, 4, 6, 7, 8, 10, 11, and 17 were found as matching well with isomer 1 of 5-caffeoylquinic acid (RT: 14.5 min), isomer 1 of *p*-coumaroylquinic (RT: 18.9 min), isomer 2 of 5 caffeoylquinic acid (RT: 22.3 min), dimer B2 of procyanidin (RT: 25.32 min), (−)-Epicatechin (RT:27.45 min), isomer 2 of P-coumaroylquinic acid (RT: 28.28 min), trimer of procyanidin (RT: 33.16 min), tetramer of procyanidin (RT: 35.7 min) and Quercetin-3-o-glucuronide (RT: 40.58 min). The most important phenolic compound was isomer 1 of *p*-coumaroylquinic acid with 208 mg ECA/kg of dry powder. In the methanolic extract of *Xylopia aethiopica* in Cameroon, Sokamte et al. [46] were identified as phenolic compounds Gallic acid, chlorogenic acid, caffeic acid, T-cinnamic acid, catechin, Epicatechin and Eugenol. The abundant phenolic compound identified by these authors was Epicathechin, who are also present in *Xylopia aethiopica* used in the present study. The differences noted in the phytochemical composition of the extracts can be due to plant age, environmental conditions and variety.

3.3. Preliminary antioxidant test

3.3.1. DPPH test

DPPH was used to assess the radical scavenging of the methanol extract of *Allium cepa* leaves and *Xylopia cepa* fruits. The results are summarized in Table 2. As presented in that Table, the antioxidant activity of onion leaves extract were no change significantly with increase concentration of extract. Their activity was different to that of BHT at all concentration. Concerning Kimba fruits extract, their antioxidant activity significantly ($P < 0.05$) increase with the concentration of extract and the high activity was found at 200 µg/mL concentration. The radical scavenging activity of this plant extract was similar at all concentration to that of BHT. Compared to *Allium cepa* leaves extract, *Xylopia aethiopica* extract exhibits the highest antiradical activity against the DPPH free radical. The variation noted may be explain by the high amount of total phenolic content in methanol extract of *Xylopia aethiopica* (15.12 ± 0.15 g EAG/100g of dry extract) against lower amount in methanol extract of *Allium cepa* (6.83 ± 0.13 g EAG/100g of dry extract). These phenolic content in *Xylopia aethiopica* have high amount of compounds for which powerful antioxidant activity were established in literature. Mendoza-Taco et al. [47] and Oscar et al. [48] also demonstrated that *Xylopia aethiopica* and *Allium cepa* are powerful free radical scavengers.

Apart from their capacity to donate hydrogen or electron, antioxidant role of phenolic compounds is also assigned to their stable radical intermediates, which limit especially fatty acids and oils oxidation from food products. According to the lipid substrate, antioxidant activity may vary widely. Hydrophilic antioxidants are more efficient in lipophilic environment providing an excellent stability against lipid oxidation due to their orientation towards the oil-air interface. In a hydrophilic environment, hydrophilic

Table 3
Ferric reducing antioxidant power of *Xylopia aethiopica* fruits and *Alium cepa* leaves extracts and theirs IC50 (mg/mL).

Extracts	Extract concentrations (μg/ml)					IC50 (mg/mL)
	25	50	100	150	200	
Xylopia aethiopica fruits	12.86 ± 0.51^{e}	57.50 ± 0.12^{d}	192.50 ± 0.71^{c}	408.50 ± 0.71^{b}	701.50 ± 2.12^{a}	12.87 ± 0.02^{b}
Allium cepa leaves	11.96 ± 0.25^{e}	20.00 ± 0.41^{d}	80.50 ± 0.71^{c}	122.00.00 ± 0.00^{b}	208.50 ± 0.70^{a}	44.90 ± 0.41^{a}
BHT	183.23 ± 0.90^{e}	370.34 ± 1.20^{d}	747.46 ± 2.40^{c}	1066.10 ± 7.19^{b}	2100.00 ± 7.19^{a}	4.93 ± 0.51^{c}

Values with different letters in the same line are significantly different at the probability threshold p< 0.05; Mean ± Standard deviation; n = 3.

Table 4
Changes in free tatty acid value (% Oleic acid) in viscera fish oil during accelerated conditions.

Samples	Day 0	Days 4	Days 8	Days 12	Days 16
VO (Control)	1.95 ± 0.16 ^{c}A	3.32 ± 0.06^{b}A	3.66 ± 0.00 ^{a}A	3.72 ± 0.01 ^{a}A	3.82 ± 0.01 ^{a}A
VO + BHT$_{200ppm}$	1.95 ± 0.16 ^{c}A	2.40 ± 0.01^{b}D	2.51 ± 0.09 ^{ab}EF	2.60 ± 0.02 ^{ab}F	2.72 ± 0.01 ^{a}HI
VO + XE$_{200ppm}$	1.95 ± 0.16 ^{c}A	2.56 ± 0.02 ^{c}C	2.68 ± 0.03 ^{ab}CD	2.79 ± 0.02 ^{ab}CD	3.14 ± 0.04 ^{a}C
VO + XE4$_{00ppm}$	1.95 ± 0.16 ^{c}A	2.55 ± 0.00 ^{b}C	2 0.62 ± 0.02 ^{ab}CD	2.75 ± 0.02 ^{ab}Cd	2.82 ± 0.01 ^{a}E
VO + XE$_{600ppm}$	1.95 ± 0.16 ^{c}A	2.52 ± 0.01 ^{b}C	2.56 ± 0.01 ^{ab}DE	2.66 ± 0.02 ^{ab}E	2.74 ± 0.00 ^{a}GH
VO + XE$_{800ppm}$	1.95 ± 0.16 ^{c}A	2.43 ± 0.01 ^{b}D	2.53 ± 0.01 ^{ab}EF	2.62 ± 0.00 ^{ab}EF	2.68 ± 0.01 ^{a}I
VO + XE$_{1000ppm}$	1.95 ± 0.16 ^{c}A	2.42 ± 0.00 ^{a}D	2.47 ± 0.01 ^{f}A	2.54 ± 0.01 ^{a}G	2.56 ± 0.03 ^{a}J
VO + AC$_{200ppm}$	1.95 ± 0.16 ^{c}A	2.63 ± 0.02 ^{b}B	2.74 ± 0.01 ^{b}B	2.82 ± 0.02 ^{b}B	3.34 ± 0.02 ^{a}B
VO + AC$_{400ppm}$	1.95 ± 0.16 ^{c}A	2.62 ± 0.02 ^{b}B	2.74 ± 0.03 ^{ab}B	2.76 ± 0.04 ^{ab}CD	2.85 ± 0.02 ^{a}D
VO + AC$_{600ppm}$	1.95 ± 0.16 ^{c}A	2.57 ± 0.01 ^{b}C	2.65 ± 0.01 ^{ab}C	2.74 ± 0.01 ^{ab}D	2.80 ± 0.01 ^{a}EF
VO + AC$_{800ppm}$	1.95 ± 0.16 ^{c}A	2.56 ± 0.01 ^{b}C	2.62 ± 0.01 ^{ab}CD	2.71 ± 0.00 ^{ab}D	2.77 ± 0.01 ^{a}FG
VO + AC$_{1000ppm}$	1.95 ± 0.16 ^{c}A	2.45 ± 0.01 ^{b}D	2.57 ± 0.01 ^{ab}DE	2.62 ± 0.01 ^{ab}EF	2.71 ± 0.00 ^{a}HI

(a-c) Means in each row with different columns are significantly (P < 0.05) different. (A-J) Means in each column with different columns are significantly (P < 0.05) different. (Control: no antioxidant; VO + BHT 200 ppm: VO: viscera fish oil containing BHT as antioxidant at concentration 200 ppm; VO + XE: viscera fish oil supplemented with *Xylopia aethiopica* extract at concentration (200ppm–1000 ppm); VO + AC: viscera fish oil supplemented with *Allium cepa* leaves extract at concentration (200ppm–1000 ppm).

antioxidants tend to dilute and act weakly against lipid oxidation [49].

3.3.2. Ferric reducing antioxidant power assay (FRAP)

The reduction capacity of the plant extracts was assessed by the FRAP assay. The FRAP assay measures the reduction capacity of the TPTZ Fe(III) complex to its ferrous TPTZ-Fe(II) form by antioxidant compound. As presented in Table 3, all the extracts act in a dose dependent manner for FRAP assay. The FRAP values varied significantly (P < 0.05) between the extracts and the similar trend were observed with those of BHT. As noted in Table 3, all sample increased their reducing ability when the concentration of extracts was increased. The highest activity was observed with methanol extract of *Xylopia aethiopica* compared to those of *Allium cepa*. The IC_{50} of *Xylopia aethiopica* was lowest (12.87 mg/mL) compared to those of onion leaves (44.90 mg/mL) and was found to exhibits the higher reduction capacity of TPTZ Fe(III) complex to its ferrous TPTZ-Fe(II) form. BHT compared to the two plant extracts was found to have a highest capacity to reduce Fe(III) complex to FE(II) form, these are confirmed by their lower IC50 (4.93 mg/mL). The high antioxidant capacity of methanol extract of *Xylopia aethiopica* in the reduction of TPTZ Fe(III) complex to its ferrous TPTZ-Fe(II) form could be attributed to their high content of phenolic, flavonoids and tannins content compared to that of *Allium cepa* leaves. These observations are in agreement with those of Sokamte et al. [46] who noted a high antioxidant activity in kimba extract compared to another plant extracts. Gulcin et al. [50] and Noriham et al. [51] demonstrated antioxidative activity on Pimpinella anisum seeds extracts and four types of Malaysian plants. The antioxidant properties of these two plants used in the present study may be attributed to their phenols content, which enable them to behave as hydrogen donors, reducing agents and quenchers of single oxygen. As mentioned by Gordon et al. [52], The radical-scavenging activity of phenolic acids depends partly on the number of electron donor hydroxyl and methoxy substituents, which increase the stability of the phenoxy radicals. Gallic acid with three hydroxyl groups and a carboxyl group is very active in reducing free radicals. The phenolic compounds naturally found in plant are very benefit for human health [53].

3.4. Effect of plant extracts on viscera fish oil oxidative stability during accelerated conditions

3.4.1. Free fatty acid (FFA) measurement

The percentage of oleic acid is the term used to measure the FFAs formed in the oil by the hydrolytic rancidity during storage [14]. It determines the oxidative damage of oil during storage or during processing as well as under varied temperature conditions. The oils with less increase in FFA during storage are more stable and show less oxidative damage. Table 4 shows evolution of FFAs in stabilized and non-stabilized viscera fish oil under accelerated conditions. Before storage, the FFA of viscera fish oil was 1.95 % oleic acid. During accelerated storage, a significant increase in FFAs value was recorded in viscera fish oil. With fish oil stabilized with the two plant extracts, increase in FFAS during storage was less as compared with non-stabilized oil samples. The FFAs during the processing decreases when the concentration of plant extracts increases. The value of FFAs are also proportional to the storage days and the oil stored

Table 5
Changes in Iodine value (g I_2/100g of oil) in viscera fish oil during accelerated conditions.

Samples	Day 0	Days 4	Days 8	Days 12	Days 16
VO (Control)	100.29 ± 0.07 ^{a}A	81.12 ± 0.52 ^{b}H	78.23 ± 0.87 ^{b}G	77.24 ± 0.52 ^{c}D	67.16 ± 0.07 ^{d}F
VO + BHT$_{200ppm}$	100.29 ± 0.07 ^{a}A	95.48 ± 0.43 ^{b}A	91.58 ± 0.67 ^{c}A	77.24 ± 0.52 ^{d}D	75.70 ± 0.62 ^{d}AB
VO + XE$_{200ppm}$	100.29 ± 0.07 ^{a}A	87.32 ± 0.89 ^{b}G	76.86 ± 0.67^{c}GH	74.72 ± 0.72^{d}EF	72.6 ± 0.79 ^{e}DC
VO + XE4$_{00ppm}$	100.29 ± 0.07 ^{a}A	87.41 ± 0.32 ^{b}G	76.01 ± 0.89 ^{b}H	73.57 ± 0.78 ^{c}G	70.77 ± 0.79 ^{d}DE
VO + XE$_{600ppm}$	100.29 ± 0.07 ^{a}A	88.84 ± 0.28 ^{b}EF	80.53 ± 1.09 ^{c}F	73.22 ± 0.01 ^{d}G	69.80 ± 0.72 ^{e}E
VO + XE$_{800ppm}$	100.29 ± 0.07 ^{a}A	89.84 ± 0.03 ^{eb}D	80.51 ± 0.55 ^{c}F	76.01 ± 0.51^{d}DE	72.60 ± 0.77 ^{e}DC
VO + XE$_{1000ppm}$	100.29 ± 0.07 ^{a}A	91.55 ± 0.38^{b}BC	80.99 ± 0.03 ^{c}F	76.88 ± 1.03 ^{d}D	74.71 ± 0.72 ^{e}BC
VO + AC$_{200ppm}$	100.29 ± 0.07 ^{a}A	87.96 ± 0.71^{b}FG	83.06 ± 0.85 ^{c}E	79.32 ± 0.01^{d}C	74.71 ± 0.72^{e}BC
VO + AC$_{400ppm}$	100.29 ± 0.07 ^{a}A	88.67 ± 0.00^{b}F	84.73 ± 0.71 ^{c}D	82.81 ± 0.21^{d}B	75.66 ± 0.61^{eb}A
VO + AC$_{600ppm}$	100.29 ± 0.07 ^{a}A	88.94 ± 0.40^{b}EF	85.62 ± 0.62^{c}CD	82.19 ± 0.09^{d}B	76.69 ± 0.76^{eb}A
VO + AC$_{800ppm}$	100.29 ± 0.07 ^{a}A	90.66 ± 0.79^{b}CD	86.67 ± 0.67^{c}BC	82.82 ± 1.04^{d}B	77.73 ± 0.64^{e}A
VO + AC$_{1000ppm}$	100.29 ± 0.07Aa	92.57 ± 0.67^{b}B	87.77 ± 0.56^{c}B	85.71 ± 0.65^{c}A	72.20 ± 2.87^{d}AB

(a-e) Means in each row with different columns are significantly ($P < 0.05$) different. (A-H) Means in each column with different columns are significantly ($P < 0.05$) different. (Control: no antioxidant; VO + BHT 200 ppm: VO: viscera fish oil containing BHT as antioxidant at concentration 200 ppm; VO + XE: viscera fish oil supplemented with *Xylopia aethiopica* extract at concentration (200ppm–1000 ppm); VO + AC: viscera fish oil supplemented with *Allium cepa* leaves extract at concentration (200ppm–1000 ppm).

Table 6
Changes in Peroxide value (meq O_2/kg of oil) in viscera fish oil during accelerated conditions.

Samples	Day 0	Days 4	Days 8	Days 12	Days 16
VO (Control)	4.69 ± 0.52 ^{d}A	13.66 ± 0.84 ^{c}A	15.19 ± 0.04 ^{b}A	16.19 ± 0.03 ^{ab}A	17.21 ± 0.04 ^{a}A
VO + BHT$_{200ppm}$	4.69 ± 0.52 ^{d}A	5.07 ± 0.08H ^{a}b	5.29 ± 0.12 ^{ab}K	5.34 ± 0.21 ^{ab}F	5.58 ± 0.43 ^{a}H
VO + XE$_{200ppm}$	4.69 ± 0.52 ^{d}A	12.07 ± 0.02 ^{c}B	12.30 ± 0.02 ^{c}C	14.28 ± 0.14 ^{b}B	15.65 ± 0.52 ^{a}B
VO + XE4$_{00ppm}$	4.69 ± 0.52 ^{d}A	9.09 ± 0.02 ^{c}CD	9.29 ± 0.02 ^{c}E	10.65 ± 0.69 ^{b}C	12.66 ± 0.48 ^{a}D
VO + XE$_{600ppm}$	4.69 ± 0.52 ^{d}A	8.12 ± 0.04 ^{c}E	9.07 ± 0.07 ^{b}F	9.38 ± 0.04 ^{b}D	11.28 ± 0.05 ^{a}EF
VO + XE$_{800ppm}$	4.69 ± 0.52 ^{d}A	7.26 ± 0.04 ^{c}F	8.36 ± 0.07 ^{b}H	9.18 ± 0.03 ^{b}D	10.86 ± 0.62 ^{a}FG
VO + XE$_{1000ppm}$	4.69 ± 0.52 ^{d}A	6.11 ± 0.00 ^{d}G	7.41 ± 0.05 ^{c}J	8.20 ± 0.05 ^{b}E	10.16 ± 0.04 ^{a}G
VO + AC$_{200ppm}$	4.69 ± 0.52 ^{d}A	12.24 ± 0.09 ^{d}B	13.31 ± 0.05 ^{c}B	14.71 ± 0.63 ^{d}B	16.43 ± 0.29^{a} ^{b}A
VO + AC$_{400ppm}$	4.69 ± 0.52 ^{d}A	9.33 ± 0.02 ^{c}C	10.74 ± 0.04 ^{b}D	11.04 ± 0.31 ^{b}C	13.29 ± 0.02 ^{a}C
VO + AC$_{600ppm}$	4.69 ± 0.52 ^{d}A	8.69 ± 0.00 ^{c}DE	9.20 ± 0.04 ^{bc}EF	9.64 ± 0.33 ^{b}D	12.76 ± 0.07 ^{a}D
VO + AC$_{800ppm}$	4.69 ± 0.52 ^{d}A	7.45 ± 0.15 ^{c}F	8.72 ± 0.08 ^{b}G	9.34 ± 0.02 ^{b}D	11.76 ± 0.06 ^{a}E
VO + AC$_{1000ppm}$	4.69 ± 0.52 ^{d}A	6.29 ± 0.04 ^{b}G	7.69 ± 0.02 ^{c}I	8.31 ± 0.07 ^{b}E	10.57 ± 0.04 ^{a}FG

(a-d) Means in each row with different columns are significantly ($P < 0.05$) different. (A-J) Means in each column with different columns are significantly ($P < 0.05$) different. (Control: no antioxidant; VO + BHT 200 ppm: VO: viscera fish oil containing BHT as antioxidant at concentration 200 ppm; VO + XE: viscera fish oil supplemented with *Xylopia aethiopica* extract at concentration (200ppm–1000 ppm); VO + AC: viscera fish oil supplemented with *Allium cepa* leaves extract at concentration (200ppm–1000 ppm).

at 16 days have the higher values. The sample stabilized with *Xylopia aethiopica* extract at 1000 ppm for 16 days compared to that stabilized with *Allium cepa* leaves extract at the same concentration for the same storage days was found to have the lowest FFA value (2.56 % oleic acid). In the same time fish oil non supplemented with antioxidant (control) was found to have the highest FFA value (3.82 % oleic acid). The plant extracts used in the present study were efficient to shut down the rate of FFA formation. Increase in FFA during storage is consistent with many previous report [12,14,15]. The lipolitic enzymes present in the fish oil mainly the lipases, often derived by microorganism, decomposed triglycerides into glycerol and free fatty acids as reported by Tenyang et al. [54]. A delay in the hydrolysis of triglycerides in the fish oil stabilized by BHT and plant extracts were revealed by the lowest FFA contains. FFAs which are not only important from the point of view of oxidation products have been reported to have direct sensory impact. It is desirable that the FFAs content of edible oils were within the limit of 0.0–3% of oleic acid. Except the negative control sample and those supplemented at 200 ppm of *Xylopia aethiopica* and *Allium cepa* leaves for 16 days of storage, all the other samples investigated presented a low value of FFAs which indicated that they were in good quality.

3.4.2. Iodine value (IV) measurement

Fish oil contains more than 60 % of unsaturated fatty acids. During processing, these compounds are susceptible to lipid oxidation. Their double bond reacts with free radicals, which results to the formation of conjugated bonds. Iodine value which is the number of grams of iodine that will react with the double bonds in 100 g of fats or oils is an important characteristic of oil or fat and it indicates the proportion of unsaturated fatty acids present. The changes in IV in viscera fish oil stabilized at different concentration of plant extract (200–1000 ppm) compared with the positive control (oil stabilized with BHT) and negative control (oil without stabilized) during an accelerated condition are presented in Table 5. No changes in IV of viscera fish oil was observed in all samples in initial day of storage ($P>0.05$). subsequently, gradual decrease was found throughout the storage and up day 12, IV of fish oil supplemented with *Xylopia aethiopica* extract at the concentration 800 ppm and 1000 ppm were comparable. The same trend was noted with samples supplemented with Allium cepa leaves extract at the concentration 400, 600 and 1000 ppm stored for 12 days. After 12 days of storage, the

Table 7
Changes in *p*-Anisidine value in viscera fish oil during accelerated conditions.

Samples	Day 0	Days 4	Days 8	Days 12	Days 16
VO (Control)	18.17 ± 0.09 ^{d}A	22.23 ± 0.08 ^{c}A	23.12 ± 0.05 ^{c}A	25.81 ± 0.28 ^{d}A	30.15± 1.02 ^{a}A
VO + BHT$_{200ppm}$	18.17 ± 0.09 ^{d}A	19.32 ± 0.08 ^{c}E	21.18 ± 0.02 ^{d}C	22.16 ± 0.07 ^{a}CD	22.53 ± 0.31 ^{a}EF
VO + XE$_{200ppm}$	18.17 ± 0.09 ^{d}A	20.65 ± 0.01 ^{c}BC	22.51 ± 0.05 ^{b}B	23.78 ± 1.35 ^{ab}B	25.13 ± 0.04 ^{a}B
VO + XE4$_{00ppm}$	18.17 ± 0.09 ^{d}A	20.22 ± 0.08 ^{c}CD	22.22 ± 0.02 ^{b}B	22.31 ± 0.07 ^{b}C	23.11 ± 0.00 ^{a}D
VO + XE$_{600ppm}$	18.17 ± 0.09 ^{d}A	20.15 ± 0.02 ^{d}D	21.11 ± 0.02 ^{c}C	22.17 ± 0.02 ^{b}CD	22.34 ± 0.02 ^{a}EF
VO + XE$_{800ppm}$	18.17 ± 0.09 ^{d}A	19.14 ± 0.09 ^{d}E	20.34 ± 0.38 ^{c}D	21.12 ± 0.02 ^{b}E	22.14 ± 0.13 ^{a}F
VO + XE$_{1000ppm}$	18.17 ± 0.09 ^{d}A	19.05 ± 0.012 ^{c}E	19.32 ± 0.40 ^{c}E	20.12 ± 0.06 ^{b}F	21.19 ± 0.02 ^{a}G
VO + AC$_{200ppm}$	18.17 ± 0.09 ^{d}A	20.76 ± 0.07 ^{d}B	22.58 ± 0.10 ^{c}B	23.79 ± 0.02 ^{b}B	25.22 ± 0.04 ^{a}B
VO + AC$_{400ppm}$	18.17 ± 0.09 ^{d}A	20.76 ± 0.63 ^{c}B	21.28 ± 0.03 ^{c}C	22.79 ± 0.02 ^{b}C	23.99 ± 0.24 ^{a}C
VO + AC$_{600ppm}$	18.17 ± 0.09 ^{d}A	20.20 ± 0.02 ^{d}CD	21.20 ± 0.04 ^{c}C	22.23 ± 0.02 ^{b}C	22.74 ± 0.09 ^{a}DE
VO + AC$_{800ppm}$	18.17 ± 0.09 ^{d}A	20.03 ± 0.03 ^{d}D	20.65 ± 0.01 ^{c}D	21.28 ± 0.01 ^{b}DE	22.33 ± 0.03 ^{a}EF
VO + AC$_{1000ppm}$	18.17 ± 0.09 ^{d}A	19.23 ± 0.00 ^{d}E	19.87 ± 0.16 ^{c}E	20.22 ± 0.04 ^{b}F	21.25 ± 0.04 ^{a}G

(a-d) Means in each row with different columns are significantly ($P < 0.05$) different. (A-G) Means in each column with different columns are significantly ($P < 0.05$) different. (Control: no antioxidant; VO + BHT 200 ppm: VO: viscera fish oil containing BHT as antioxidant at concentration 200 ppm; VO + XE: viscera fish oil supplemented with *Xylopia aethiopica* extract at concentration (200ppm–1000 ppm); VO + AC: viscera fish oil supplemented with *Allium cepa* leaves extract at concentration (200ppm–1000 ppm).

higher IV (87.77 gI_2/100 g of fat) was found in the sample supplemented with *Allium cepa* leaves at the concentration of 1000 ppm, while the lower was found in oil without supplemented with antioxidant. The activity of BHT at the days 4 and 8 of storage was far from those of plant extracts, however, at the day 12 and 16, the activity of BHT was lowest than that of *Allium cepa* leaves. Compared to *Xylopia aethiopica* fruits, *Allium cepa leaves* exhibited the high antioxidant activity. Commonly, storage leads to a reduction in insaturation, thus indicating a decrease in double bonds. A decrease in IV throughout the storage period is consistent with the decrease in double bonds as oil oxidized. The rate of decrement IV in oil without BHT and plant extracts were significantly higher ($P < 0.05$) than that of supplemented oils, this is due to the addition of antioxidant which have significantly reduced the alteration of double bonds contains in fish fatty acids. It appears in the present study that in all concentration, the plant extracts have effectively delay the oxidation of fish oil unsaturated fatty acids. These study result were in agreement with the report of Womeni et al. [13] which stated that the decrease in IV in palm olein after storage shows relatively higher oxidation.

3.4.3. Peroxide value (PV) measurement

The peroxide value (PV) of an edible oil is an indicator of freshness as viewed through oxidative degradation. It is directly related to storage time and found an important parameter to access the oxidative stability of oil. Table 6 shows the variation of BHT and extracts from *Xylopia aethiopica* and *Allium cepa* leaves on PV of viscera fish oil during accelerated conditions. In this Table results shows that the PV of both stabilized and non-stabilized viscera fish oil samples at the initial time (day 0) of storage was 4.69 meqO_2/kg oil. A significant increase in PV was recorded as days of storage increases and decreases as the concentration of plant extracts increases. Viscera fish oil without antioxidant served as negative control and was found to have the higher PV during storage time. During storage, fish oil supplemented with BHT was found to have the lowest PV. The increase in PV throughout storage can be due to the formation of hydroperoxides, which are the primary products of oxidation. Addition of plant extracts to fish oil make them more resistant to oxidation and this rise in resistant caused a reduction in the gradient of the increase in PV. The lesser amount of deterioration in fish supplemented with natural antioxidant might be due to their bioactive secondary metabolite responsible to prevent lipid oxidation. These results are in line with those observed by Kedir et al. [55], who showed that PV of palm oil stabilized with selected plant extracts increases slowly when the oils are supplemented with high concentration of plant extract. Based on the recommendation of FAO/OMS [56], the maximum PV of edible oil is 10 meq O_2/kg of oil and the higher PV indicated the more oxidized oil. In the present study, all samples were found to present a PV lower than 10 meq O_2/kg of oil. After 16 days of storage, the PV of oil supplemented with *Xylopia aethiopica* at 800 and 1000 ppm and those supplemented with *Allium cepa* leaves at 1000 ppm exhibited the same and the lowest PV around 5 meq O_2/kg of oil, which indicates the good quality of these fish oil.

3.4.4. P-anisidine value analysis

Anisidine analysis is used to determine the amount of aldehyde produced by hydroperoxide breakdown, primarily 2-alkenals and 2,4-dienals. The quality of oil can be determined by evaluating the absorbance at 350 nm of the solution. The effect of synthetic antioxidant and natural antioxidant on the production of aldehyde in viscera fish oil during accelerated conditions was presented in Table 7. The *P*-anidine value of initial samples was 18.7 mL isoctan/g of oil. After that, the p-AV of all samples increased drastically as the storage period increased, but slowly when the concentration of plant extracts increased. After 16 days of storage, the less p-AV were found with samples supplemented at 1000 ppm of plant extracts. The plant extracts and BHT significantly retarded the oxidation of viscera fish oil compared to negative control. The plant extracts and BHT during the storage time exhibited the same trend, confirming the efficiency of plant extracts used in the present study in inhibiting the formation of secondary oxidation products in fish oil due to phenolic compounds present in plant extracts that contribute a hydrogen atom to peroxy radicals, and to create constant hydroperoxides. These results are in accordance with those of Womeni et al. [13] who reported that the addition of soursop flowers extract in palm olein inhibit the high product of *p*-anisidine in these oil.

Table 8
Changes in TOTOX value in viscera fish oil during accelerated conditions.

Samples	Day 0	Days 4	Days 8	Days 12	Days 16
VO (Control)	$27.55 \pm 1.13^{e}A$	$49.55 \pm 1.78^{d}A$	$55.51 \pm 0.04^{c}A$	$58.05 \pm 0.42^{b}A$	$65.1 \pm 0.83^{a}A$
VO + BHT$_{200ppm}$	$27.55 \pm 1.13^{d}A$	$29.52 \pm 0.09^{d}H$	$31.77 \pm 0.21^{c}J$	$32.75 \pm 0.36^{b}H$	$33.70 \pm 1.17^{a}G$
VO + XE$_{200ppm}$	$27.55 \pm 1.13^{d}A$	$44.80 \pm 0.02^{c}B$	$47.12 \pm 0.09^{c}C$	$52.33 \pm 1.06^{b}B$	$56.43 \pm 1.08^{a}B$
VO + XE4$_{00ppm}$	$27.55 \pm 1.13^{e}A$	$38.40 \pm 0.14^{c}CD$	$40.80 \pm 0.07^{c}E$	$43.61 \pm 1.46^{b}C$	$48.43 \pm 0.96^{a}D$
VO + XE$_{600ppm}$	$27.55 \pm 1.13^{e}A$	$36.51 \pm 0.11^{d}E$	$39.26 \pm 0.17^{c}F$	$40.93 \pm 0.11^{b}DE$	$44.90 \pm 0.09^{a}E$
VO + XE$_{800ppm}$	$27.55 \pm 1.13^{e}A$	$33.66 \pm 0.17^{d}F$	$37.06 \pm 0.24^{c}G$	$39.49 \pm 0.09^{b}EF$	$43.87 \pm 1.39^{a}E$
VO + XE$_{1000ppm}$	$27.55 \pm 1.13^{e}A$	$31.28 \pm 0.14^{d}G$	$34.34 \pm 0.51^{c}I$	$36.52 \pm 0.04^{b}G$	$41.51 \pm 0.06^{a}F$
VO + AC$_{200ppm}$	$27.55 \pm 1.13^{e}A$	45n.24 $\pm 0.12^{d}B$	$49.20 \pm 0.21^{c}B$	$53.21 \pm 1.29^{b}B$	$58.08 \pm 0.04^{a}B$
VO + AC$_{400ppm}$	$27.55 \pm 1.13^{e}A$	$39.43 \pm 0.67^{d}C$	$42.76 \pm 0.04^{c}D$	$44.87 \pm 0.56^{b}C$	$50.57 \pm 0.30^{a}C$
VO + AC$_{600ppm}$	$27.55 \pm 1.13^{d}A$	$37.56 \pm 0.00^{c}DE$	$39.60 \pm 0.04^{b}F$	$41.51 \pm 0.65^{b}D$	$48.27 \pm 0.05^{a}D$
VO + AC$_{800ppm}$	$27.55 \pm 1.13^{e}A$	$34.08 \pm 0.24^{a}F$	$37.31 \pm 0.00^{c}G$	$38.90 \pm 0.00^{b}F$	$44.78 \pm 0.16^{a}E$
VO + AC$_{1000ppm}$	$27.55 \pm 1.13^{d}A$	$31.81 \pm 0.07^{c}G$	$35.25 \pm 0.10^{b}F$	$36.84 \pm 0.09^{b}G$	$41.90 \pm 0.84^{a}F$

(a-e) Means in each row with different columns are significantly ($P < 0.05$) different. (A-J) Means in each column with different columns are significantly ($P < 0.05$) different. (Control: no antioxidant; VO + BHT 200 ppm: VO: viscera fish oil containing BHT as antioxidant at concentration 200 ppm; VO + XE: viscera fish oil supplemented with *Xylopia aethiopica* extract at concentration (200ppm–1000 ppm); VO + AC: viscera fish oil supplemented with *Allium cepa* leaves extract at concentration (200ppm–1000 ppm).

3.4.5. TOTOX value measurement

The TOTOX value is a common approach to determining the resistance to oxidative rancidity of edible oils. These of the viscera fish oil stabilized with plant extracts, BHT and non-stabilized are presented in Table 8. In the initial days of storage (Day 0), the TOTOX value in all samples were the same and lowest (27.55). After initial day of storage, the TOTOX value showed a significant ($P < 0.05$) difference between the oil supplemented with BHT, those supplemented with plant extracts and the non-stabilized oil during accelerated storage conditions. The high value of TOTOX was found in non-stabilized fish oil when oil supplemented with synthetic and natural antioxidants were less oxidized. The resistance toward oxidation is proportional to the concentration of plants extract added. The TOTOX value of oil supplemented with BHT were significantly lower than those supplemented with plant extracts. Throughout the plant extracts, the variation might be due to the type of plant, the composition and the amount of phenolic compounds present in each plant extract. Similar conclusion was made by Womeni et al. [13] when studing the effect of soursop flowers extract in palm olein during accelerated conditions. Flores et al. [57] mentioned that the good quality vegetable oils have TOTOX values less than 4. The TOTOX value obtained in this study are greater than the proposed limit.

4. Conclusion

In this study, it is clear that the quality of viscera fish oil was significantly affected during accelerated storage as revealed by assessing the oil quality indices. *Xylopia aethiopica* fruits and *Allium cepa* leaves used contains good quantities of phenolic compounds and *Allium cepa* compared to *X. aethiopica* contains high proportions of quercetin and it derivatives. These phenolic compounds can be used as natural antioxidant for oil stabilization. These plant extracts are a potent free radical scavenger and ferric reducer. Added in viscera fish oil during accelerated conditions, these plant extracts showed a significant improvement in oil quality. With BHT they were significantly retarded fish oil alteration and maintains the oil quality. They were recommended to the oil and fat industries as an alternative antioxidant to synthetic antioxidant. *Allium cepa* appear to be the easiest and best antioxidant that can be used to stabilized fish oil during storage due to their high content of quercetin and its derivatives which are easily dissolve in oil and better prevent the lipid oxidation.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

N. Tenyang: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **B.Z. Haman:** Methodology, Investigation. **A.J. Tawai:** Methodology, Investigation. **Z.G. Tabanty:** Methodology, Investigation. **G. Douka:** Methodology, Investigation. **R. Ponka:** Writing – review & editing, Formal analysis, Data curation. **H.M. Womeni:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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