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Extraction, fractionation and assessment of antioxidant activities of active components of *Aframomum sceptrum* seeds

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A simple procedure of extraction, fractionation and assessment of organoleptic and antioxidant properties of *Aframomum sceptrum* seeds was carried out, and the results were compared with a currently used synthetic antioxidant, butylated hydroxy toluene (BHT). Extraction was carried out using Soxhlet extractor and fractionation of the ethanolic extract was accomplished using vacuum liquid chromatography (VLC). Various fractions of the ethanolic extract were obtained but similar fractions were pooled together to yield six fractions (F1-F6) as guided by thin layer chromatography (TLC) analysis. Fractions obtained were subjected to sensory, chemical, spectroscopic and storage assessment in order to evaluate their antioxidant potentials. Sensory characterization and antioxidant storage tests showed that fractions F4 and F5 had non-bitter taste and yellow colour in contrast to bitter taste and dark brown colour of the crude extract. Statistical analysis (P= 0.05) showed that the antioxidant effectiveness (AE) of the fractionated extracts F4 and F5 were 62.57 and 59.13% for groundnut oil and 64.50 and 62.91% for red palm oil, respectively, and compared favourably with the standard synthetic antioxidant used. The fractions can be used to extend the shelf life of food products.

Key words: Spices, fractionated extracts, antioxidant effectiveness, active components, sensory and spectroscopic characteristics.

INTRODUCTION

Free radicals can be generated by metabolic pathways in the body tissues or in food systems. Many synthetic chemicals such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) though very effective as antioxidants, have been known to have toxic and carcinogenic effects on humans (Yoshihara et al., 2010). Synthetic antioxidants may result in liver swelling and influence liver enzyme activities (Yoshihara et al., 2010). Therefore, considering these reports and the present trends towards naturally preserved food products, herbs and spices have been targeted as sources of natural antioxidants (Kimura et al., 2010; Brewer, 2011). The use of these plant materials as natural antioxidants for food, cosmetics and other application becomes necessary because of food safety issues: (1) safety, since they are part of food man has been eating for thousands of years

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(Bongoni et al., 2013); (2) good carry through effectiveness, since they survive processing operations; (3) their use is not guided by legislatory rules (Cohen and Ernst, 2010) and (4) their source is renewable. Seeds of *Aframomum sceptrum* have been shown to possess antioxidant activity superior to some currently used synthetic antioxidants (Auqustyniak et al., 2010). However, the seed and its crude extracts were bitter, a quality which could limit their application in food systems. The constraint serves as impetus to this study. Investigations carried out on fractionation and assessment of phytochemicals and antioxidant activities of fractionated active components of the *A. sceptrum* seeds were reported in this study.

MATERIALS AND METHODS

Seeds of *A. sceptrum* used in this study were purchased from a local market in Agbor, Delta State of Nigeria. They were identified and authenticated at the Botany Department of Delta State University, Abraka, Nigeria. The dried seeds were carefully removed from the pod manually. The removed seeds were cleaned - up of all extraneous materials and adhering particles by putting in a cotton sack, rubbed, then put in a tray and winnowed. The seeds were air-dried at 25°C for 2 days, to further reduce the moisture content. The air-dried seeds were pulverized into a fine powdery form using a Warren blender. Groundnut oil was purchased from local processor in Bida, Niger State, Nigeria. Palm oil was purchased from Nigerian Institute For Oil Palm Research (NIFOR) oil mill at Benin City, Edo State, Nigeria. Standard antioxidant used was BHT (Aldrich Chem. Co). All other chemicals used were of analytical grade.

Preparation of ethanolic extract of *A. sceptrum*

Solvent extraction of the seeds of *A. sceptrum* was done using the method stated in Mircea and Isabel (2015). An amount of 10 g of finely ground *A. sceptrum* powder was extracted with 100 ml of 95% ethanol in Soxhlet extractor for 8 h. The crude extract was evaporated to remove the solvent, and the percentage yield of the extract was determined.

Fractionation of *A. sceptrum* extracts using vacuum liquid chromatography (VLC)

Fifteen grams (15 g) of ethanolic crude extract was dissolved in 150 ml of ethanol (95%), followed by the addition of 120 g of pre-treated silica gel (Na ethanoate-treated gel). The mixture was mixed until thorough blend was obtained, then air-dried at 30°C in air-oven, and milled manually to obtain “pre-adsorbed sample”. Five grams (5 g) of the pre-adsorbed sample was subjected to vacuum liquid chromatography (VLC) on silica gel using a mobile phase (50ml each) gradient in toluene: ethyl ethanoate: methanol. Fractions were monitored by thin layer chromatography (TLC). Similar fractions were pooled together to produce six fractions (F1-F6), evaporated to dryness and kept in the dark for subsequent analysis (Perqola and Werz, 2010).

Characterization of crude and fractionated extract of *A. sceptrum*

Sensory characteristics of crude and fractionated extracts of *A. sceptrum* were determined following the procedure of Bongoni et al. (2013). For determination of chemical compounds, standard phytochemical screening procedure was carried out according to the method of Marcia et al. (2011). Total phenol was determined according to the method of Lu et al. (2011). The reducing power of crude and fractionated extract was determined using the method of Canabady-Rochelle et al. (2015). Other assays used to determine the antioxidant potentials of the extract include; DPPH spectrometric assay by the method of Chandrasekar et al. (2014), hydrogen peroxide scavenging activity by the method of Graf and John (2015), superoxide radical scavenging activity by the method of Shuhui et al. (2010), nitric oxide radical scavenging activity by the method of Anguo et al. (2003), hydroxyl radical scavenging activity according to the method of Isaksen and Dalsoren (2011), total flavonoid content by the method of Chen and Dingrong (2012), free fatty acid (AOCS, 2012) and peroxide value (AOCS, 2011). Ultra violet (UV) visible absorption spectra of crude and fractionated extracts were recorded on a UV- visible double beam recording spectrophotometer. Infrared (IR) spectral characteristic of crude and fractionated extracts were recorded on a Fourier- Transform- IR spectrophotometer.

Antioxidant incorporation and evaluation of antioxidant activities of *A. sceptrum*

The extracts and standard antioxidant were added into the oils at recommended concentration of 20 mg/100 g (0.02%) by direct addition at 50°C and stirred using BHT (standard antioxidant) as positive control and oils without additive as negative control. Antioxidant activities of the fractionated extracts were evaluated in treated oils by Schaal oven test. Treated samples were stored in an oven and maintained at a temperature of 63°C. Negative control was also placed under the same condition of storage as the treated sample. Oxidative stability was monitored by measuring peroxide values at regular intervals of seven days during the storage period (Akinmoladun et al., 2010). Decrease in the rate of formation of peroxides was used as a measurement of antioxidant activities (Akinmoladun et al., 2010).

Statistical analysis

Data obtained from evaluation of antioxidant activities at different concentrations using changes in peroxide values with time were subjected to statistical analysis and results obtained were tested for significant difference at 5% level using analysis of variance (ANOVA). Means were separated using Turkey’s test (Lowry, 2013).

RESULTS AND DISCUSSION

Phytochemical components of seeds, crude and fractionated extracts of *A. sceptrum*

Phytochemical screening of the seeds, crude extracts and fractions of *A. sceptrum* showed the presence of alkaloids, flavonoids, phenols, anthraquinones, tannins, saponins and glycosides (Table 1). This result is in agreement with earlier report of Marcia et al. (2011) on the class of secondary chemical compounds present in the seeds of *A. sceptrum* and plants belonging to the botanical family Zingiberaceae. The determination of the dominant secondary metabolites in the fractions is

Table 1. Phytochemical components in the seeds, crude and fractionated extracts of *A. sceptrum.*

Phytochemical	Seed	ETH	Fractions					
			1	2	3	4	5	6
Tannin	+	+	+	+	+	+	+	+
Saponin	++	++	+	+	+	-	-	+
Alkaloids	+++	++	++	++	++	-	+	++
Flavonoid	++	+++	+	+	+	+++	+++	+
Flavonol	+	+++	-	-	+	+++	++	-
Anthraquinone	+	+	-	-	-	-	+	-
Glycoside	+	+	+	+	+	+	+	+
Phenol	+	++	+	+	+	++	++	+

+++ Highly positive; ++ moderately positive; + slightly positive; - negative (not present).

necessary for structural elucidation of active compounds responsible for antioxidative activities. Flavonoid and its derivative flavonol were dominantly present in F4 as well as F5 with trace amount of anthraquinones. Flavonoids and their derivatives have been known to be the most active polyphenolic antioxidant (Marcia et al., 2011; Hussein et al., 2013). The effectiveness of flavonoid in retarding lipid oxidation in fat containing foods is related to their ability to act as free radical acceptors (Dai and Mumper, 2010) or as chelators of metal ions. The chelation by flavonoids is due to *ortho*-dihydroxy (3, 4-dihydroxyl) group on the β- ring in their chemical structure (Dai and Mumper, 2010). Flavonoid is also known to exhibit antimicrobial properties, while anthraquinone is known for its laxative properties. In line with Osbourn et al. (2011) and Erukainure et al. (2011), saponin and glycosides were detected in the seeds and fractions of *A. sceptrum.*

Sensory characteristics of crude and fractionated extracts

The active components of the seeds of *A. sceptrum* were separated into six fractions using vacuum liquid chromatography (VLC) and their sensory characteristics are shown in Table 2. The crude ethanolic extract was a brown viscous liquid with pungent and bitter taste. The dominating bitter taste is as a result of alkaloids present in *A. sceptrum* (Marcia et al., 2011). The various fractions obtained from VLC techniques were of varying degree of yellow colour with different kinds of taste, which could have been as a result of the dominant secondary metabolites. Fractions F4 and F5 were light-yellow and sunset yellow respectively in colour with non-bitter taste in contrast to dark colour and bitter taste associated with crude extracts. Such fractionated extracts will be good for preventing lipid peroxidation in food systems. Objectionable taste and unappealing colour are some of the characteristics that limit the application of antioxidants from natural sources (Karlen et al., 2011).

Table 2. Sensory characteristics of crude and fractionated extracts of *A. sceptrum.*

Antioxidant	Sensory characteristics	
	Colour	Taste
ETH	Dark brown	Bitter
STEAM	Brown	Bitter
F1	Golden yellow	Tasteless
F2	Reddish yellow	Slightly bitter
F3	Brown	Bitter
F4	Light yellow	Tasty (sweet)
F5	Sun-flower yellow	Slightly bitter
F6	Light yellow	Bitter

Spectroscopic analysis of crude and fractionated extracts of *A. sceptrum* - ultraviolet (UV) spectral characteristics of crude and fractionated extracts

Crude ethanolic extracts were separated into six fractions as guided by thin layer chromatography. Their UV spectral as extrapolated from UV curves is shown in Table 3. The crude extract (ETH) had strong absorption at 235 nm. The most potent fractions (F4 and F5) had strong absorptions at 240 and 210 nm respectively. Strong absorptions (primary and secondary) in the ultra violet region by the samples are diagnostic feature of unsaturation or non-bonded electron in the absorbing molecules (Akinmoladun et al., 2010). Free electron is a pre-requisite for antioxidant activity (Auqustyniak et al., 2010). In addition to this high wavelength of maximum absorption (λmax), the samples were characterized with large magnitude of molar extinction coefficient (ε). The magnititude of a molar coefficient for a particular absorption is directly proportional to the probability of the particular electronic transition, the more the given transition, the larger the extinction coefficient (Mariani et al., 2012). While both primary and secondary λmax of fraction F4 were higher than that of the standard antioxidant, BHT, the spectroscopic characteristics of the standard antioxidant is comparable to that of fraction F5.

Table 3. UV-spectral characteristics of crude and fractionated extracts of *A. sceptrum.*

Sample	λ_1 (nm)	A_1 (nm)	ε_1 (Lmole^{-1}cm^{-1})	λ_2 (nm)	A_2(nm)	ε_2(Lmole^{-1}cm^{-1})
ETH	235	2.821	19,219	263	2.506	17,835
F4	240	3.261	19,644	298	2.815	17,600
BHT	230	2.685	18,126	286	1.965	17,143
F1	217	2.203	5,620	222	0.581	2,264
F5	210	2.82	16,663	228	1.768	10,638
F3	226	2.143	3,642	272	1.398	2,533
F6	214	1.553	3,120	232	1.42	2,412
F2	218	2.166	3,200	262	1.02	2,760

Table 4. IR- spectral characteristics of the crude and fractionated extracts of *A. sceptrum.*

Sample	Vmax (cm^{-1})				
F1	3323 (b)	1288 - 1050 (b)	706 (w)	721 (w)	
F2	3466 (b)		780 (w)		
F3	3387 (b)	1208 (b)	816 (m)	780 (w)	640 (w)
F4	3364 (b)	1830 (s)	1452 (s)	965 (sh)	
F5	3449 (b)	1568 (sh)	1334 (s)	901 (v)	
F6	3359 (b)				
ETH	3386 (b)	1620 (sh)	1152 (s)	924 (w)	826 (w)

S = Strong; m = medium; w = weak; v = variable; b = broad; sh = sharp.

Infrared (IR) spectral characteristics of crude and fractionated extracts

Functional groups were assigned to IR – absorption peaks using functional group –IR frequency chart adapted from Neil (2005). Peaks of diagnostic value as extrapolated from IR curves (not shown) were identified and selected based on IR- functional group- antioxidant potential. The functional groups are phenolic hydroxyl group (≈3390 c m^{-1}), unsaturated/free electron potential (1600-1670cm^{-1}), carboxylate ions (1762 c m^{-1}), aromatic nuclei (800-900 c m^{-1}). The presence of peaks in the region and their intensities are indices of antioxidant potentials of crude and fractionated extracts. These properties are more pronounced in ETH, F4 and F5. The functional groups are the building blocks of antioxidant compounds (Table 4).

Antioxidant activities of *A. sceptrum* fractions in groundnut and red palm oils

Antioxidant activities of fractions of *A. sceptrum* and standard antioxidant (BHT) in the oils were evaluated. The peroxide values of the oil samples were determined when the oils were fresh and periodically at 7 days interval for 35 days as shown in Figures 1 and 2. Fractions F4 and F5 had the highest antioxidant effectiveness (AE) of 62.57 and 59.13% in groundnut oil, while 64.50 and 62.91% was observed in red palm oil respectively (Figures 3 and 4). The dominant secondary metabolites in the fractions are flavonoids, its derivative (flavonol) and anthraquinones. Flavonoid is a class of polyphenols with effective antioxidant activities (Hussein et al., 2013). Fraction F2 in which alkaloids are dominant had lower AE (32.62%) in groundnut oil and AE (42.42%) in red palm oil respectively when compared to activities of F4 and F5, showing that alkaloids are not the major contributors to the antioxidant properties of *A. sceptrum.* The antioxidant effectiveness (AE) of the fractions F4 and F5 were similar to that of BHT. The results indicated a comparable (p=0.05) effectiveness of fractions F4 and F5 to the reference antioxidant.

Relationship between phenolic content, reducing power and absorbance of extracts of *A. sceptrum*

Phenolic content, reducing power and absorbance of the fractions of *A. sceptrum* are shown in Table 5. Correlation (r=0.880) exists between phenolic content and reducing power. This suggested that the mode of antioxidant action of phenols in *A. sceptrum* is more of electron-transfer or abstraction of hydrogen from hydroxylic group of phenolic compounds than of resonance stability by phenyl moiety. There was also a slight relationship (r=

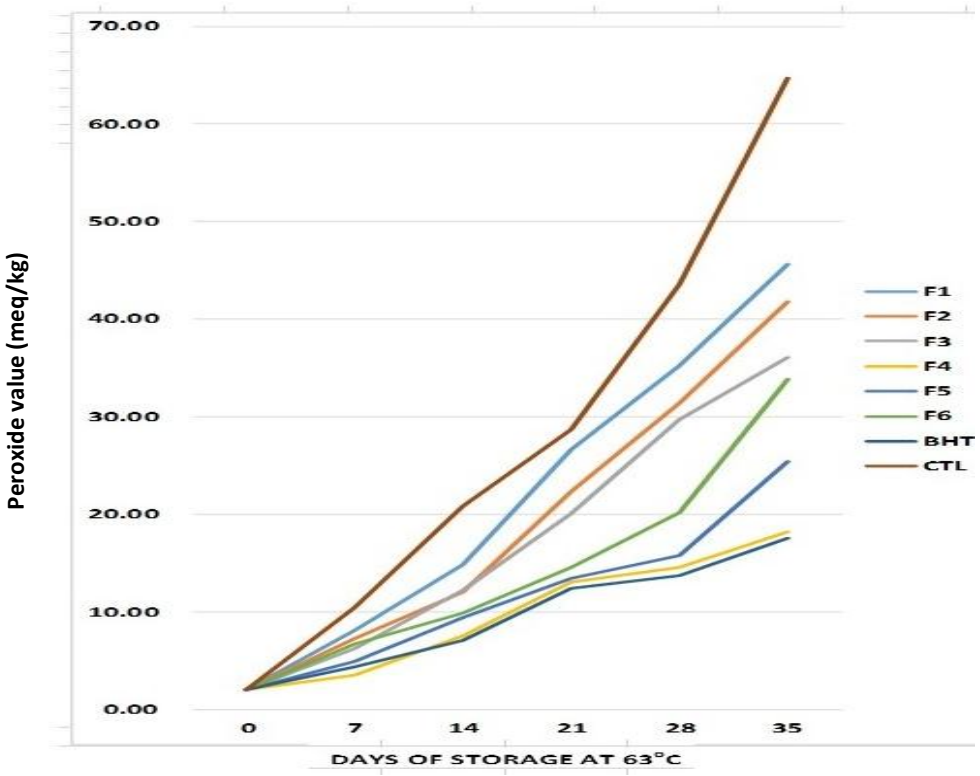

Figure 1. Antioxidant activity of fractions of *A. sceptrum* and BHT in groundnut oil at 0.02% concentrations.

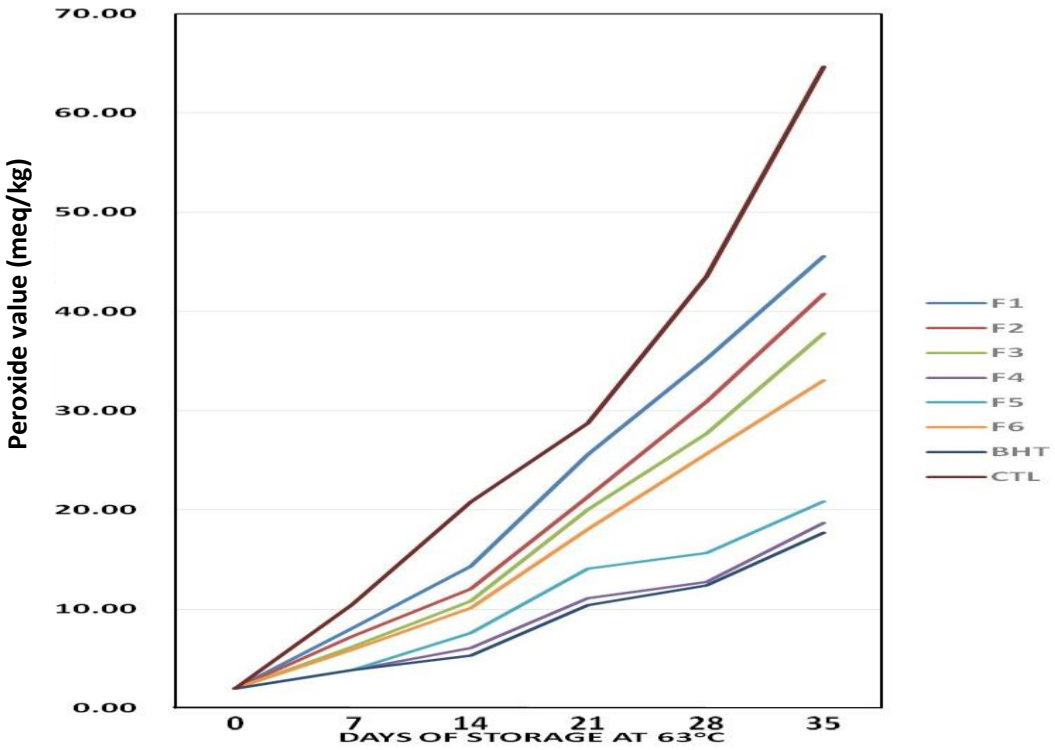

Figure 2. Antioxidant activity of fractions of *A. sceptrum* and BHT in red palm oil at 0.02% concentration.

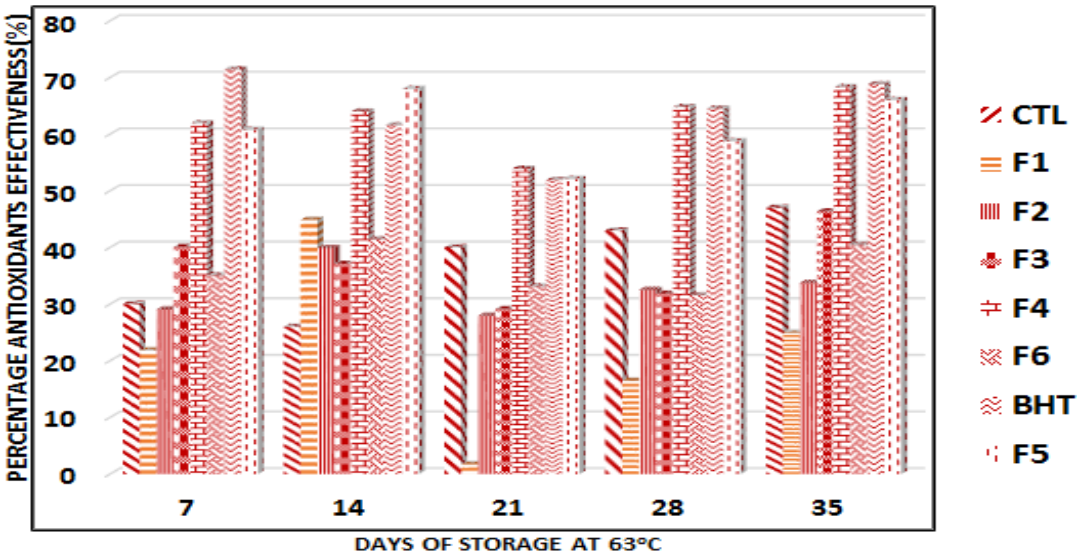

Figure 3. Percentage antioxidant effectiveness of fractions of *A. sceptrum* and BHT at 0.02% concentrations in groundnut oil.

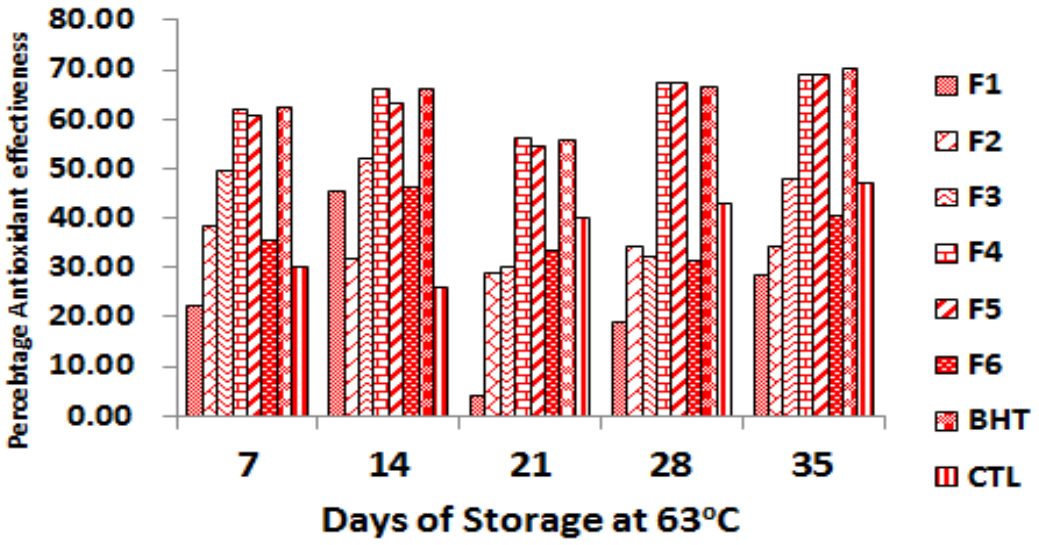

Figure 4. Percentage antioxidant effectiveness of fractions of *A. sceptrum* and BHT at 0.02% concentrations in red palm oil.

Table 5. Phenolic content, reducing power and absorbance at λmax of extracts of *A. sceptrum*.

Sample	Phenolic content (g/g ascorbic acid equivalent)	Reducing power	Absorbance at λmax
F1	0.700	0.010	2.300
F2	1.370	0.150	2.820
F3	1.430	0.121	2.810
F4	1.850	0.183	3.350
F5	1.420	0.140	2.600
F6	0.720	0.015	2.320
ETH	1.830	0.161	3.200

0.650) between total phenol content and absorbance at primary λmax. This implied that the phenolic compounds in the crude extracts and fractionated extracts are of variable qualities (Mouming et al., 2014).

Conclusion

The presence of bioactive compounds in *A. sceptrum* is an affirmation of its use in the management of various ailments, and consumption of this spice in combination of other foodstuff can help meet the nutritional needs of the individual. The fractionation of viscous, dark brown and bitter extracts of *A. sceptrum* yielded a non-viscous, yellow and tasty extract which compared favourably with the one of the currently used synthetic antioxidants. This fraction is recommended for use in food preservation.

Conflict of interests

The authors did not declare any conflict of interest.

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