sciendo

DOI: 10.2478/auoc-2023-0014

Ovidius University Annals of Chemistry Volume 34, Number 2, pp. 104 - 111, 2023

Xylopia aethiopica HPLC-DAD polyphenol profiling and antioxidant status from South-South region of Nigeria

Joel OKPOGHONO*,[1] Ejovi OSIOMA,[2] Sandra Oghenekohwo METIE,[3] Udoka Bessie IGUE,[3] Endurance Fegor ISOJE,[4] and Solomon Ugochukwu OKOM[1]

[1]*Department of Biochemistry, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria*
[2]*Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria*
[3]*Department of Chemical Sciences, Novena University, Ogume, Delta State, Nigeria*
[4]*Department of Chemistry, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria*

Abstract. To fill nutrient gaps, supplements are employed in the field of nutrition. The spice *Xylopia aethiopica* grows wild in many African countries and has been used as flavouring for soups. This study evaluates the polyphenols and antioxidant profile of different fractions of *X. aethiopica* methanol extract. Powdered sample of *X. aethiopica* (250 g) was suspended in methanol (1.5 litre) for 48 hours and the extract was concentrated at 45 °C using water bath to obtain methanol crude extract which was fractionated into *n*-hexane (n-Hex), chloroform (Chl), diethyl ether (DEE), *n*-butanol (n-Bu) and aqueous fractions using liquid- liquid partition separation technique. Antioxidant properties were investigated using standard methods and the polyphenols were estimated using high performance liquid chromatography with diode-array detector (HPLC-DAD). Results revealed that the DEE fraction had significantly ($p < 0.05$) high total phenol content (TPC), proanthocyanidins, anthocyanins, 1,1-diphenyl-2-picrylhidrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC) as compared with other fractions. The highest luteolin and taxifolin content was detected in n-Hex fraction. Polyphenols found in DEE fraction in the established HPLC-DAD assay were *p*-anisic acid (0.07 ng/g), caffeic acid (0.56 ng/g), vanillic acid (0.91 ng/g), salicylic acid (1.64 ng/g), gallic acid (3.09 ng/mL), ferulic acid (3.64 ng/g), *p*-coumaric acid (5.15 ng/g), sinapinic acid (6.63 ng/g) and protocatechuic acid (24.89 ng/g). The different fractions of *X. aethiopica* displayed various polyphenols potential with probable antioxidant activity, which may be useful in neutralizing free radicals and the treatment of chronic inflammatory associated metabolic ailments, such as obesity and diabetes.

***Keywords*:** HPLC-DAD; luteolin; polyphenols; taxifolin; *Xylopia aethiopica*.

1. Introduction

Plants-based products have been employed for centuries in the management of various diseases [1]. The health promoting potentials of plant originated natural products have been related to a variety of chemical constituents and structures of secondary metabolites in the genus in which the botanicals belong [2]. Dietary enrichments are any products consumed in addition to a diet. Dietary supplements consist of vitamins, minerals, fish oils, herbal teas, and more. Plant-based foods contain a variety of antioxidants and phytonutrients, including polyphenols, flavonoids, catechins and phytoestrogens [2].

In terms of structure and biological characteristics, plants are a significant source of active natural compounds. The deterrence of malignancy and cardiac diseases has been associated with the ingestion of spices, fresh fruits, vegetables, or teas rich in natural antioxidants [3]. The protective effects/potential of plant products are due to the presence of several components/active ingredients [4-10], which have distinct approach of action based on the polarity of extracting solvents [1].

Xylopia aethiopica belonging to the genus *Xylopia* (*Annonaceae*), is a tropical evergreen tree bearing aromatic seeds. *Xylopia aethiopica* grows in tropical Africa. It is usually called “negro pepper” or “Ethiopia pepper”. In Nigeria, it is in the neighborhood known as Kimbaa (Hausa), Uda (Igbo) and Erínje or Èèrù (Yoruba). The dried fruit is used as tonic in the cure of bronchitis, asthma, infertility, wounds, arthritis and rheumatism, post-natal pains and dysenteric conditions [11].

The medicinal effects of the plant with respect to its ethnobotanical claims could be accredited to the presence of various bioactive compounds in the botanical [12, 13]. However, several factors that could influence the potencies and the biological functions of these bioactive compounds had been identified through various systematic studies; these include processing, handling and extraction techniques [14]. Furthermore, biological activities of bioactive compounds may be inclined generally by the chemical characteristics of the solvent used for the extraction [15]. The contemporary technique for extracting phenolics uses organic solvents. The identification of active compounds with biological

* Corresponding author. *E-mail address*: okpoghono@gmail.com (Joel Okpoghono)

© 2023 Joel Okpoghono et al.
This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDerivs Licence (https://creativecommons.org/licenses/by-nc-nd/3.0/).

properties may be aided by novel analytical techniques. Quantification of a general estimation of the concentration of phenolic compounds is made possible, mostly through spectrophotometry techniques. However, more accurate studies are focused on identifying specific phenolic classes using sensitive detectors like mass spectrometry (MS) or high-performance liquid chromatography (HPLC) or gas chromatography (GC) [15]. HPLC combined with DAD is a highly modern technique used for the qualitative and quantitative evaluation of polyphenols. The HPLC is a potent method for the analysis, identification, and assessment of natural compounds like polyphenols [15].

This study was anticipated to investigate the HPLC-DAD polyphenol profile and the antioxidant potentials of different fractions of *X. aethiopica* from South-South part of Nigeria. The results of the study would aid in assessing *X. aethiopica* polyphenols potential in relation to the ethnomedicinal uses of *X. aethiopica* by the people of Southern part of Nigeria were the plant is highly cultivated. All across the world, especially in Africa were *X. aethiopica* is been used for soup preparation and concoctions will be enlightened on the possibility of the intake of the polyphenol enrich medicinal plant supplement. The study might also help enlighten people on the better solvent for the fractionation of polyphenol active ingredients of *X. aethiopica* from South-South part of Nigeria.

2. Experimental

2.1. Materials and reagents

X. aethiopica fruits were purchased from Igbudu market Warri Delta State. The dried *X. aethiopica* was identified with voucher specimen number (UBHdt/SN/059) at the University of Benin herbarium.

The reagents n-hexane, chloroform, diethyl ether, n-butanol, Folin-Ciocalteau were all purchased from BDH chemical laboratory England. Potassium persulphate was purchased from Sigma chemical company, London, England. All other reagents used were of analytical grade.

2.2. Extraction of compounds for phytochemical analysis

1.0 L of methanol was used to suspend a portion of 250 g of dried powder *X. aethiopica* for 48 hours in large brown bottles with recurrent shaking. The crude methanol extract was concentrated in a 45 °C water bath after being filtered using muslin fabric. Afterword, the crude extract was fractionated using standard procedures.

2.3. Fractionation

Liquid-liquid partition separation was used to separate the crude *X. aethiopica* extract into different fractions. The crude extract (50 g) was reconstituted with 250 mL of each solvent (n-Hex, Chl, DEE, n-Bu) and 250 mL of water 1:1 (v/v) in a separating funnel and then rocked vigorously. The mixture was let to stand in the separator funnel for 30 minutes with each solvent until a fine separation line clearly delineated the supernatant from the sediment before it was successively eluted. The process was repeated three times in order to get adequate quantity for each fraction. The n-Hex, Chl, DEE, n-Bu and the aqueous residue fractions were evaporated to dryness in a water bath to obtain five fractions. Analyses were conducted on the fractions. 1 g of each fraction was reconstituted in 9 mL aqueous tween 80 (5%) to stabilized of the solution. Different concentrations (100 µg/mL, 200 µg/mL, 300 µg/mL and 400 µg/mL) from the crude extract and fractions (n-Hex, Chl, DEE, n-Bu fractions) were prepared. The solutions were used to assay for the antioxidant properties. The fraction with the highest antioxidant properties was subjected to HPLC-DAD polyphenol profiling.

2.4. Determination of antioxidant activities in the various fractions

TPC determination. The procedure outlined by Dewanto et al. [16] was followed in order to determine TPC. In 100 µL of Folin-Ciocalteau reagent and 6 mL of distilled water, 0.5 mL of samples (crude extract and fractions) at different concentrations was dissolved in test tubes. After 1 minute of vortexing, 2 mL of 15% Na_2CO_3 solution was added to each test tube, and the mixture was vortexed once more for 30 seconds. With distilled water, the solutions were diluted up to 10 mL. UV-Vis spectrometer (Digital Aqua Mate AQ8000 UV-VIS; Thermo Scientific Coimbatore, India) was used to measure the samples' absorbance at 750 nm after one hour and thirty minutes. Gallic acid was used as the standard, and concentrations between 100 and 1000 mg/mL were used to create the calibration curve. TPC was calculated as mg gallic acid equivalent/100 g of dry weight (d.w.).

2.5. TFC determination

The colorimetric aluminium chloride method was used to measure TFC [17]. A sample (crude extract and fractions) at different concentrations (0.5 mL) was combined with 0.5 mL of 2% $AlCl_3$ solution in methanol. After 10 minutes, absorbance was measured at 415 nm against blank sample. The calibration curve was made using rutin at concentrations ranging from 100 to 1000 mg/mL as the standard. TFC was considered as mg rutin equivalent/100 g of dry weight.

2.6. DPPH free radical assay

The radical scavenging capacity of the sample (crude extract and fractions at different concentration; 100-400 µg/mL) against DPPH free radical was estimated using the method described by Ursini et al. [18]. Fifty microliters (50 µL) of each test sample was diluted with 3 mL ethanol and mixed with 3 ml DPPH solution. The reaction mix was incubated in dark for 30 minutes. The solution was read at 517 nm against a blank. The following formula was used to get the radical scavenging percentage:

$$\text{Radical scavenging percentage} = \frac{A_0 - A_1}{A_0} \times 100$$

where: A_0 was the absorbance of DPPH solution (control) and A_1 was the absorbance of the sample.

2.7. FRAP assay

The ability to reduce ferric ions was examined using the method designated by Benzie and Strain [19]. The FRAP solution was prepared by mixing 300 mM sodium

acetate buffer (pH 3.6), 10.0 mM tripyridyltriazine (TPTZ) solution and 20.0 mM $FeCl_3 \cdot 6H_2O$ solution in a ratio of 10:1:1 in volume. 0.5 mL of samples at different concentrations was then added to 3 mL of FRAP reagent. The reaction mix was permitted to stand at 37 °C for 30 minutes. The increase in absorbance at 593 nm was measured. The standard curve was linear between 50 and 400 µM $FeSO_4$. Results are expressed in µM Fe (II)/g dry mass.

2.8. ABTS radical scavenging assay

ABTS was determined according to method of Re et al. [20].

The ABTS cation solution was formed by the reaction of ABTS stock solution (5 mL) and 2.45 mM potassium persulphate ($K_2S_2O_8$) solution (5 mL), stored in the dark at room temperature for 16 hours. Samples (0.5 mL) at different concentrations was added to 4.5 ml ABTS radical cation solution in test tubes, and allowed to incubate at room temperature for 6 min. The sample absorbance was recorded at 734 nm. Blanks (control) were also done in each assay. The ABTS percentage inhibition was calculated using the formula below:

$$\text{Inhibition percentage} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 = the absorbance of the control, A_1 = the absorbance of the sample.

2.9. TAC assay

TAC was estimated by Prieto et al. method [21]. Sample crude extract and fractions (0.1 mL) at different concentrations was added to1 mL of reagent solution (28 mmol/L Na_3PO_4, 4 mmol/L ammonium molybdate and 0.6 mol/L H_2SO_4) in test tubes and then incubated at 95ºC for 90 minutes. The mixture was allowed to cool at room temperature and read at 695 nm against blank. Gallic acid (100 - 1000 mg/ml) was used as the standard. The values were expressed as mg gallic acid equivalent per gram dry weight.

2.10. Determination of polyphenols

Polyphenol in fractions (*in vitro*) were determined.

2.10.1. Proanthocyanidin. Proanthocyanidins (condensed tannins) were determined using the method of Sun et al. [22].

The test tube was filled with 0.5 mL of samples at different concentrations, 3 mL of 4% vanillin solution in methanol, and 1.5 mL of concentrated HCl. After 15 minutes of standing time, the combination was tested for absorbance at 500 nm using methanol as a reference. The calibration curve was created using a catechin solution. The amount of total condensed tannins using a catechin calibration curve was expressed as mg catechin /g dw.

2.10.2. Anthocyanins. Based on the technique outlined by Iland et al. [23], the total anthocyanins were estimated. 200 µL of the sample (crude extract and fractions at different concentrations) was combined with 3.8 mL of 1 M HCl, in different test tubes. The mixture was then let to stand at room temperature for three hours. Using 1 M HCl as the reference solution, the absorbance of the acidified, diluted sample was measured at 520 nm.

2.10.3. Determination of flavanonols: dihydroquercetin (taxifolin) and flavones (luteolin). To determine taxifolin and luteolin, Struchkov et al. technique was used [24]. To 0.1 mL of crude extract and fractions, 0.3 mL of 5% $NaNO_2$ and 5 mL of water were added. After 5 min, 1.5 mL of 1M NaOH was added, followed by 5 µL of acetic acid (20%) and 0.2 mL of 10% $AlCl_3$ solution. The UV spectrum was calibrated between 300 and 600 nm against the blank. Taxifolin and Luteolin concentration in mg/g was calculated according to the following formula:

$$X\ (mg/g) = \frac{10\ x\ VxA}{p\ x\ E}$$

where: X – total content of taxifolin or luteolin; V = 8 mL; A – absorption at 517 nm for luteolin, and 507 nm for taxifolin; p – sample volume (mL); E - taxifolin specific absorbance = 134.5 at 507 nm, or luteolin specific absorbance = 63.9 at 517 nm.

2.10.4. Polyphenol profiling. The fraction with the highest antioxidant properties (DEE fraction) was chosen for HPLC-DAD analysis polyphenol profiling. This was done using a Shimadzu Prominence Auto Sampler HPLC (SIL-20A; Shimadzu, Kyoto, Japan) armed with photo-diode array detector SPD-M20A. For peak detection and identification, the following phenolic acid standards (purity > 98%): *p*-anisic acid, caffeic acid, vanillic acid, salicylic acid, gallic acid, ferulic acid, *p*-coumaric acid, sinapinic acid and protocatechuic acid were used. The mobile phase contains 1% aqueous acetic acid solution (Solvent X) and acetonitrile (Solvent Y), and 2 mL/min was adjusted as the flow rate. The sample injection volume was 5 µL, and the column's temperature was maintained at 28 ºC. By adjusting the ratio of solvent X to solvent Y, a gradient elution was carried out. In 55 minutes, the solvent B: solvent A: 10:90 ratio of the mobile phase was restored, and then another 10 minutes were given to pass before injecting another sample. The entire analysis time per sample was 65 minutes. According to the substances examined, the HPLC chromatogram was detected using a photo DAD at three different wavelengths: 272, 280, and 310 nm. Based on their retention time and by spiking with the standards under the same circumstances, compounds were identified. The integrated peak area was measured for sample quantification, and the content was computed.

Polyphenols content were calculated as follows:

$$\text{Concentration of polyphenols (ng/g)} = \frac{\text{Peak area}}{\text{Standard peak area}} \times \text{Standard concentration}$$

2.11. Statistical analysis

Results were stated in mean ± SD and mean bars. All data were analyzed using Analysis of variance (ANOVA). Significant difference between means were determined at $p < 0.05$ confidence level using lest significant difference (LSD). The SPSS version 22.0 was used for statistical analysis.

3. Results and discussion

The hydrogen atom transfer (HAT), the electron transfer (ET), and the combination of both HAT and ET are the three main mechanisms that govern the antioxidant approaches used in this study. HAT measures an antioxidant's capacity to squelch free radicals through hydrogen donation, while ET assesses an antioxidant's capacity to transfer one electron to squelch radicals, metals, and carbonyls. The Folin-Ciocalteu reagent measures the TPC through the ET mechanism, and FRAP is measured using the ET mechanism. Two free radicals that are frequently employed to evaluate the *in-vitro* antioxidant capacity of samples are ABTS and DPPH [20]. Total phenolic content, FRAP, and radical scavenging activity as determined by DPPH and ABTS were used to define the antioxidant capacity [20]. A general mechanism employed by antioxidants in eliciting their protective effects has been through donating electrons to reactive oxygen species, thus making them more stable [25-28]. In this study, the results enable us to categorize the *X. aethiopica* fractions based on their antioxidant measurements. The ABTS, DPPH, FRAP, TFC, TPC, and TAC of crude extract and *X. aethiopica* fractions are shown in Table 1 and 2.

Table 1. Total flavonoids FRAP, ABTS and total phenol content of *X. aethiopica* different fractions and crude extract.

	Sample conc. (μg/mL)	**Total Flavonoids (mg rutin /g d.w.)**	**FRAP (μM Fe(II)/g)**	**ABTS (% inhibition)**	**Total Phenol (mg GAE/g d.w.)**
Chloroform fraction	100	130.50±19.30 [a]	35.80±7.45 [a]	26.74 ± 5.50 [a]	169.30±29.30 [a]
	200	137.15±45.55 [b]	37.50±13.10 [a]	30.60 ±11.50 [a]	170.95±35.20 [a]
	300	145.30±34.50 [c]	50.89±13.45 [b]	40.50 ± 12.31 [b]	177.53±22.80 [b]
	400	152.50±22.50 [d]	60.47±9.30 [c]	50.10 ± 6.75 [c]	184.80±24.29 [c]
DEE fraction	100	167.20±17.15 [e]	55.72±9.51 [b]	33.50±5.35 [a]	210.36±15.70 [d]
	200	175.10±21.90 [f]	62.00±8.85 [c]	45.35±4.07 [b]	215.20±16.15 [d]
	300	190.50±11.20 [g]	73.57±7.55 [d]	50.45±3.40 [b,c]	227.85±29.97 [e]
	400	203.00±14.20 [h]	80.72±9.53 [f]	60.51±2.52 [d]	235.40±12.53 [f]
n-Hexane fraction	100	151.20±27.25 [d]	40.40±17.65 [a]	28.20±4.85 [a]	184.56±42.25 [c]
	200	167.95±38.35 [e]	50.20±18.50 [b]	38.55±6.22 [a,b]	200.10±29.30 [e]
	300	165.20±20.35 [e]	60.65±12.52 [c]	45.69±2.04 [b]	211.16±33.45 [d]
	400	172.55±40.50 [e,f]	73.50±11.15 [d]	52.65±7.53 [b,c]	220.15±14.15 [f]
n-Butanol fraction	100	91.50±10.90 [i]	38.15±12.50 [a]	23.29±5.04 [a]	140.06±13.31 [g]
	200	104.20±10.45 [j]	48.50±16.75 [b]	28.51±8.60 [a]	144.30±11.30 [g]
	300	108.60±22.70 [j]	57.55±12.50 [b,c]	37.48±3.84 [a,b]	152.00±15.81 [h]
	400	112.50±23.20 [k]	70.60±17.10 [d]	45.48±8.70 [b]	164.83±16.26 [i]
Aqueous fraction	100	62.50±5.50 [l]	28.58±8.80 [e]	20.56±5.50 [e]	108.00±7.37 [d]
	200	65.16±10.16 [l]	40.01±5.20 [a]	28.09±7.20 [a]	113.80±10.20 [d]
	300	73.50±5.20 [m]	48.56±6.50 [b]	38.58±9.67 [a,b]	118.96±10.70 [f]
	400	82.40±9.10 [n]	60.12±2.59 [c]	45.47±13.20 [b]	123.80±13.25 [f]
Crude extract	100	222.25±20.50 [o]	80.55±12.06 [f]	52.20±10.17 [c]	310.00±33.70 [g]
	200	228.20±24.50 [o]	101.05±23.98 [g]	55.01±05.51 [b,c]	320.85±24.26 [h]
	300	235.80±27.70 [p]	108.50±13.59 [g,h]	70.10±7.10 [f]	327.80±31.50 [i]
	400	241.50±22.85 [p]	115.55±16.40 [h]	80.30±11.30 [g]	336.35±23.55 [j]

Triplicates values were represented in mean ± standard deviation. Mean values of the same column with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether.

Table 2. Total antioxidant capacity (TAC) and DPPH of X. *aethiopica* different fractions and crude extract.

	Sample conc. (μg/mL)	**TAC (mg GAE/g d.w.)**	**DPPH (% inhibition)**
Chloroform fraction	100	432.15 ± 33.00 [a]	60.78±1.14 [a]
	200	441.50 ±14.40 [b]	64.68±3.78 [a]
	300	446.50 ± 24.30 [b]	70.21±12.45 [a,b]
	400	454.10 ± 29.10 [c]	79.94±9.50 [c]
DEE fraction	100	630.50 ± 50.46 [d]	80.55±5.53 [c]
	200	635.30 ± 30.78 [d]	86.41±9.15 [c]
	300	639.40 ± 46.90 [e]	94.86±12.50 [d]
	400	641.50 ± 16.06 [e]	110.24±5.37 [e]
n-Hexane fraction	100	530.40 ± 64.20 [f]	71.29±2.32 [b]
	200	550.30 ± 36.12 [g]	75.89±6.52 [b]
	300	560.50 ± 40.25 [h]	80.50±5.15 [c]
	400	565.20 ± 33.50 [h]	91.41±7.10 [d]
n-Butanol fraction	100	423.57 ± 81.65 [i]	67.35±14.38 [a]
	200	438.30 ± 53.62 [a]	70.14±20.60 [a,b]
	300	448.85 ± 45.60 [b]	80.79±10.60 [c]
	400	455.35 ± 36.50 [c]	85.35±10.34 [c]

	Sample conc. (µg/mL)	TAC (mg GAE/g d.w.)	DPPH (% inhibition)
Aqueous fraction	100	294.40 ± 12.60 [j]	43.51±4.77 [f]
	200	300.25 ± 13.10 [j]	46.32±3.30 [f]
	300	320.50 ± 25.80 [k]	55.41±19.55 [a,g]
	400	330.60 ± 10. 37 [l]	60.55±11.90 [a]
Crude extract	100	705.25 ± 20.90 [m]	106.01±26.20 [i]
	200	715.30 ± 87.05 [n]	115.11±17.23 [j]
	300	720.00 ± 23.63 [n]	123.96±11.45 [k]
	400	729.50 ± 55.70 [o]	132.70±15.41 [l]
BHT	100	440.80 ± 49.90 [b]	52.54±2.60 [g]
	200	450.50 ± 58.00 [c]	61.47±9.92 [a]
	300	456.45 ± 36.13 [c]	63.73±6.08 [a]
	400	459.20 ± 35.85 [c]	73.73 ± 9.52 [b]

Triplicates values were represented in mean ± standard deviation. Mean values of parameters with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether, BHT = butylated hydroxytoluene.

Significant increase was observed in the TFC, FRAP, ABTS, TPC, DPPH radical scavenging activity and TAC of the crude extract when compared to the different fractions (n-Hex, Chl, DEE, n-Bu) and BHT (a synthetic antioxidant). Since 1954, BHT has served as antioxidants in diets for humans, and they may currently be the most widely used antioxidants [3]. Despite the widespread use of synthetic antioxidants, the food industry is pushing for natural antioxidants to take their place since customers are increasingly favoring them because they are not only more affordable but also more environmentally friendly. However, as the concentration of the sample increases (100-400 µg/mL) the antioxidant property increases. Trends of the antioxidants TPC, TFC, FRAP and ABTS were as follows: crude extract > DEE fraction > n-Hex fraction > Chl fraction > n-Bu fraction > aqueous fraction. The inhibition by the *X. aethiopica* fractions was in concentration-dependent manner.

Notably, the antioxidant trends of the crude extract and fractions showed that using the DEE solvent caused more antioxidant compounds to be extracted (or leached out). Therefore more bioactive components are extracted from the *X. aethiopica* using DEE than in other solvents. This could be due to the level of polarity of the solvents utilized during the fractionation procedure. Phytochemicals, particularly the polyphenols, for example, are more soluble in less polar substances than water. This is consistent with a study by Naczk and Shahidi [29], which found that enhancing the phenolic solubility of plant materials was mostly dependent on the polarity of the solvents.

It's suggested that *X. aethiopica* may be useful in the handling of diseases associated with high levels of free radical. However, the *X. aethiopica* DEE fraction has a higher potential of scavenging DPPH and ABTS radicals compared to other fractions.

The fractions and extract of *X. aethiopica* also significantly inhibited the formation of reactive oxidants as demonstrated in the FRAP (Table 1). From the results, it is proposed that *X. aethiopica* contain bioactive chemicals that may have donated electrons to Fe^{3+}, thus reducing the ferric complex to the ferrous form. It is further suggests that the reductive effect of *X. aethiopica* on Fe^{3+} may largely depend on the presence of phenols, and flavonoids existing in the fractions (Table 1). Antioxidants compounds such as phenol and flavonoids have been well documented to possess very strong antioxidant activity in many studies [30, 31]. Thus, the high TAC in the fractions and crude extract of *X. aethiopica* (Table 2) has shown that *X. aethiopica* possess an array of antioxidant activity. The DEE fraction was more potent as the sample concentration increases from 100 to 400 µg/mL. In this study, the antioxidant activity values quantified through DPPH were higher than the ones obtained by ABTS and FRAP. This is consistent with the result of Chaves et al. [32] who compared the antioxidant activity by the three methods (DPPH, FRAP, ABTS). According to the authors, antioxidant activity measurements made using DPPH were higher than those made using ABTS and FRAP, and these measurements varied between species. Knowing the technique to use is necessary to choose species from a pool with better antioxidant activity [32]. The study of woody species in arid regions of Mexico by Wong-Paz et al. [33] revealed that the antioxidant activity values measured by DPPH were higher than those obtained by ABTS and that the values of the two techniques differed between species.

According to Khalaf et al. [34], the stable free radical DPPH, which can absorb an electron or a hydrogen atom to form a stable diamagnetic molecule, is frequently employed to measure the radical-scavenging capacity of antioxidant chemicals. Polyaromatic hydrocarbon cations, some of which have been connected to carcinogenesis, are examples of cellular free radicals [35]. Superoxides and hydroxyls, which are also created in significant quantities by numerous physiological and metabolic processes, are other *in situ* radicals. As a result, an essential assay for determining antioxidant activity that breaks chains is radical scavenging. The products that may scavenge DPPH in a test tube may likewise scavenge polyaromatic hydrocarbon cations and other radicals in a living organism [35].

Micronutrients called polyphenols are produced naturally in plants. Although they are included in many supplements, it is also simple to incorporate foods like fruits, vegetables, teas, and spices to add them to the diet. Proanthocyanidins, anthocyanins, luteolin and taxifolin contents are shown in Figures 1-4. The results showed that the DEE fraction had significantly ($p < 0.05$) high proanthocyanidins and anthocyanins as compared with other fractions (Figure 1 and 2).

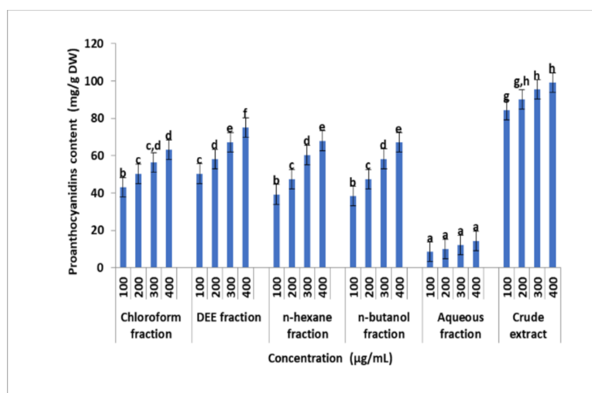

Figure 1. Proanthocyanidins content of *X. aethiopica* different fractions and crude extract. Mean bar with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether.

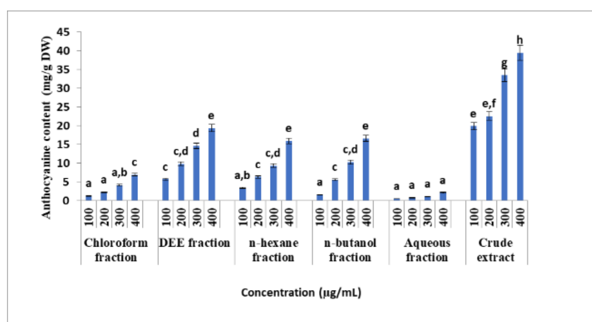

Figure 2. Anthocyanins content of *X. aethiopica* different fractions and crude extract. Mean bar with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether.

The highest luteolin and taxifolin content was detected in n-Hex fraction (Figure 3 and 4). Trends of luteolin and taxifolin content were as follows; crude extract > n-Hex fraction > DEE fraction > Chl fraction > n-Bu fraction > aqueous fraction.

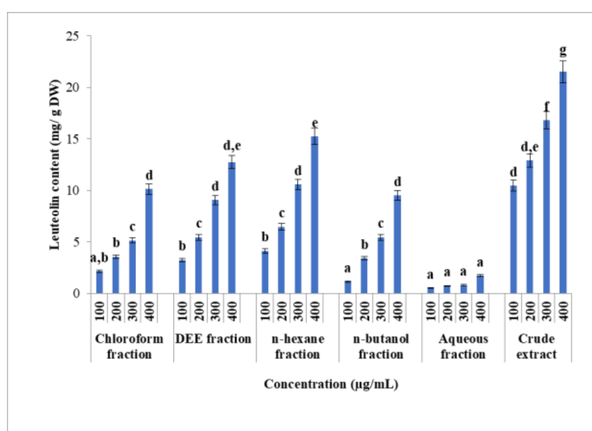

Figure 3. Luteolin content of *X. aethiopica* different fractions and crude extract. Mean bar with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether.

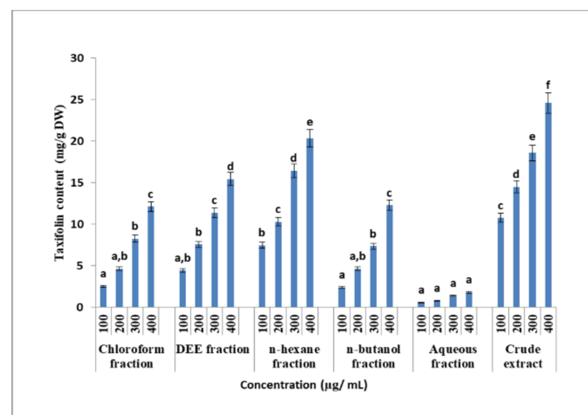

Figure 4. Taxifolin content of *X. aethiopica* different fractions and crude extract. Mean bar with different superscript letter differ significantly at $p < 0.05$. DEE = diethyl ether.

The HPLC polyphenols content and chromatogram of *X. aethiopica* DEE fraction are shown in Figure 5 and 6.

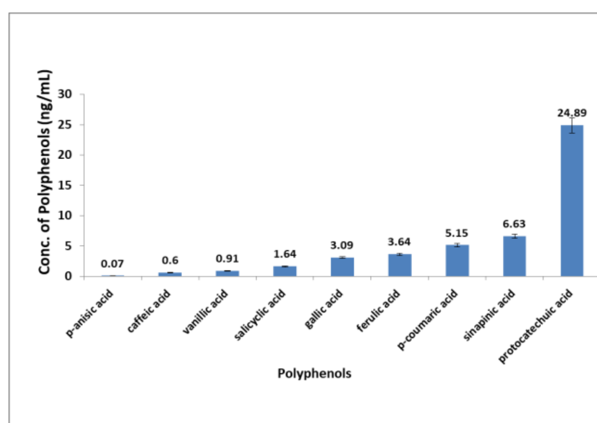

Figure 5. HPLC-DAD polyphenol contents of *X. aethiopica* DEE fraction.

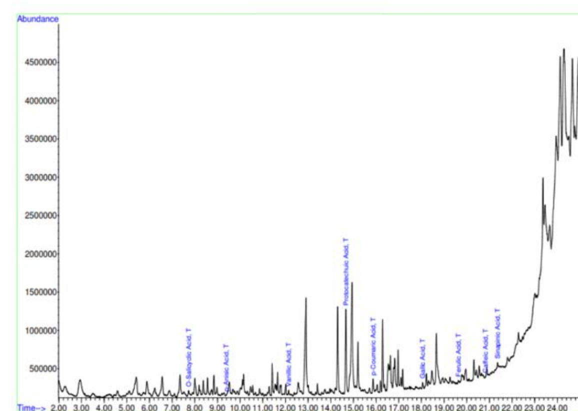

Figure 6. Chromatogram of polyphenol contents of *X. aethiopica* DEE fraction.

Polyphenols found in DEE fraction were *p*-anisic acid (0.07 ng/g), caffeic acid (0.56 ng/g), vanillic acid (0.91 ng/g), salicylic acid (1.64 ng/g), gallic acid (3.09 ng/g), ferulic acid (3.64 ng/g), *p*-coumaric acid (5.15 ng/g), sinapinic acid (6.63 ng/g) and protocatechuic acid (24.89 ng/g). The observed increase in protocatechuic acid and sinapinic acid when compared with other polyphenol compounds could be as a result of their polar

hydroxyl group. Study have shown that polarity increases extraction yield, and antioxidant accomplishment of plant, and this could be attributed to the high affinity of polyphenol compounds towards more polar solvents as compared to non-polar ones [15]. In addition, phenolic acids like gallic and vanillic acids can be found in large quantities in plants. Gallic acid, which is essentially a subordinate polyphenolic metabolite, is a well-known natural antioxidant. Polyphenols antioxidant properties are significant for improving brain health and function [36]. By scavenging free radicals, they facilitate the reduction of brain cell damage [36].

4. Conclusions

The study offered new information on the features of the fractionating solvents (such as polarity, extractive power, etc.), which were necessary to get the desired bioactive compounds. The established simple and reliable HPLC-DAD method in this investigation was successfully applied for the quantitation of the polyphenol compounds in *X. aethiopica*. The results also showed that the *X. aethiopica* fractious have strong antioxidant properties with more priority to DEE. This might be due to antioxidant compounds, especially the polyphenols which are more soluble in less polar solvents. Therefore, it is advised that the food industry may benefit from isolating each individual polyphenol. This could help to reduce body metabolic inflammations, prevent food spoilage caused by lipid oxidation, and serve as potential agents to provide functional benefits in food and human health applications.

Conflict of interest

There is no conflict of interest to declare by the authors.

References

[1]. J. Okpoghono, F.I. Achuba, B.O. George, Effect of solvent differential on the antioxidant properties, and phytochemical compounds of *Monodora myristica*, Sokoto Journal of Medical Laboratory Science 3 (2018) 88 - 100.

[2]. C.J. Camilo, C.F. Alves Nonato, F.F. Galvão-Rodrigues, W.D. Costa, G.G. Clemente, M.A.C. Sobreira Macedo, F.F. Galvão Rodrigues, J.G.M. da Costa, Acaricidal activity of essential oils: a review, Trends in Phytochemical Research 1 (2017) 183-198.

[3]. B.O. George, J. Okpoghono, Antioxidant activity of water, ethanol and diethyl ether extracts of *Monodora myristica* and *Syzygium aromaticum*, Direct Research Journal of Public Health and Environmental Technology 2 (2017) 30-35.

[4]. P. Onakurhefe, F.I. Achuba, B.O. George, J. Okpoghono, Effect of *Elaeis guineensis* (Jacq) leaf extracts on crude oil-induced genotoxicity in Wistar albino rats, Scientific African 7 (2020) e00280.

[5]. G.O. Omoike, B.O. George, E.O. Ugwu, J. Okpoghono, Changes in the antioxidant properties of *Ocimum africanum* leaves aqueous extract from the southeastern region of Nigeria after in vitro gastrointestinal digestion, International Journal of Food Properties 25 (2022) 2000-2018.

[6]. E.O. Ugwu, B.O. George, G.O. Omoike, J. Okpoghono, Bioaccessibility of polyphenols and flavonoids in *Ocimum africanum* leaves extract, Uniport Journal of Engineering and Scientific Research 6 (2021) 78-84.

[7]. A.A. Anigboro, Antioxidant effect of aqueous seed extract of *Aframomum sceptrum* (*Kschum*) on brain and kidney of malaria infested mice, Nigerian Journal of Environmental Sciences and Technology 14 (2019) 102-106.

[8]. J. Okpoghono, F.I. Achuba, B.O. George, Influence of *Monodora myristica* extracts on lipid profile of rats fed crude petroleum oil contaminated catfish diet, Sokoto Journal of Medical Laboratory Science 3 (2018) 19-27.

[9]. J. Okpoghono, B.O. George, F.I. Achuba, U.B. Igue, S.U. Okom, A.A. Seigha, L.C. Ugwuka, K.I. Ayogoi, Impact of crude petroleum oil and *Monodora myristica* on membrane bound ATPases and erythrocyte osmotic fragility *in-vivo,* Uniport Journal of Engineering and Scientific Research 6 (2021) 65-77.

[10]. H.E. Kadiri, The ameliorating effects of honey on some biochemical parameters on rats exposed to cyanide, Biokemistri 30 (2018) 13-20.

[11]. T.C. Fleischer, M.L.K. Mensah, A.Y. Mensah, G. Komlaga, S.Y. Gbedema, H. Skaltsa, Antimicrobial activity of essential oils of *Xylopia aethiopica*, African Journal of Traditional, Complementary and Alternative Medicines 5 (2008) 391 - 393.

[12]. B. Kivcak, T. Mert, Quantitative determination of alpha-tocopherol in *Arbutus unedo* by TLC-densitometry and colorimetry, Fitoterapia 72 (2001) 656–661.

[13]. S. Macdonald, P.D. Preuzier, M. Autolovich, K. Robards, Phenolic content and antioxidant activity of olive oil extracts, Food Chemistry 73 (2001) 73-84.

[14]. S.O. Nwozo, B.J. Oso, B.E. Oyinloye, Effect of heat on antioxidant activity of some tropical leafy vegetables, Nigerian Journal of Basic and Applied Sciences 23 (2015) 93-101.

[15]. M.F. Sun, C.L. Jiang, Y.S. Kong, J.L, Luo, P. Yin, G.Y. Guo, Recent advances in analytical methods for determination of polyphenols in tea: A comprehensive review, Foods 11 (2022) 1425.

[16]. X. Dewanto, K. Wu, K. Adom, R.H. Liu, Thermal processingenhances the nutritional value of tomatoes by increasingtotal antioxidant activity, Journal of Agricultural and Food Chemistry 50 (2002) 3010–3014.

[17]. M.A. Ebrahimzadeh, S.J. Hosseinimehr, A. Hamidinia, M. Jafari, Antioxidant and free radical scavenging activity of *Feijoa sallowiana* fruits peel and leaves, PharmacologyOnLine 1 (2008) 7-14.

[18]. F. Ursini, M. Maiorino, P. Morazzoni, A. Roveri, G. Pifferi, A novel antioxidant (IdB 1031)

affecting molecular mechanisms of cellular, Free Radical Biology and Medicine 16 (1994) 547-53.

[19]. I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, Analytical Biochemistry 239 (1996) 70 -76.

[20]. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS decolorization assay, Free Radical Biology and Medicine 26 (1999) 1231-1237.

[21]. P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Analytical Biochemistry 269 (1999) 337-341.

[22]. H. Sun, W. Wu, J. Guo, R. Xiao, L. Zheng, G. Zhang, Effects of nickel exposure on testicular function, oxidative stress and male reproductive dysfunction in *Spodoptera litura* Fabricius, Chemosphere 148 (2016) 178-87.

[23]. P.G. Iland, W. Cynkar, I.L. Francis, P.J. Williams, B.G. Coombe, Optimization of methods for the determination of total and red free glycosyl-glucose in black grape berries of *Vitis vinifera*, Australian Journal of Grape and Wine Research 2 (1996) 171−178.

[24]. P. Struchkov, V. Beloborodov, V. Kolkhir, I. Voskoboynikova, A. Savvateev, Comparison of spectrophotometric methods of total flavonoid assay based on complex formation with aluminum chloride as applied to multicomponent herbal drug angionorm, Journal of Pharmaceutical Negative Results 9 (2018)1-7.

[25]. L.I. Ekakitie, J. Okpoghono, O.C. Orororo, O.A. Ekakitie, Ameliorative prowess of bee honey in the tissues of rats administered aluminum nitrate, Scientific African 12 (2021) e00782.

[26]. B.O. George, B. Atalor, J. Okpoghono, Role of vitamin E, vitamin A and aqueous extract of *Aframomum sceptrum* in mice, infected with malaria parasites, Nigerian Journal of Nutritional Sciences 40 (2019) 113-117.

[27]. E.J. Otuaga, J. Okpoghono, B.O. George, Proximate composition, phytochemicals and antioxidant status of Banga soup (*Elaeis guineensis* extract), FUPRE Journal of Scientific and Industrial Research 4 (2020) 64-74.

[28]. O.E. Ejueyitsi, B.O. George, A.A. Anigboro, J. Okpoghono, Assessment of frequency and consumption pattern of some indigenous soups in Delta State, Tropical Journal of Natural Product Research 6 (2022) 592-598.

[29]. M. Naczk, F. Shahidi, Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis, Journal of Pharmaceutical and Biomedical Analysis 41 (2006)1523-1542.

[30]. K.W. Chan, S. Iqbal, N.M.H. Khong, D. Ooi, M. Ismail, Antioxidant activity of phenolic-saponin rich fraction prepared from defatted kenaf seed meal, LWT - Food Science and Technology 56 (2014) 181–186.

[31]. T.P. Yin, L. Cai, Y. Xing, J. Yu, X.J. Li, R.F. Mei, Z.T. Ding, Alkaloids with antioxidant activities from *Aconitum handelianum*, Journal of Asian Natural Products Research 18 (2016) 603–610.

[32]. N. Chaves, A. Santiago, J.C. Alías, Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used, Antioxidants 9 (2020) 76.

[33]. J.E. Wong-Paz, J.C. Contreras-Esquivel, R. Rodríguez-Herrera, M.L. Carrillo-Inungaray, L.I. López, G.V. Nevárez-Moorillón, C.N. Aguilar, Total phenolic content, *in vitro* antioxidant activity and chemical composition of plant extracts from semiarid Mexican region, Asian Pacific Journal of Tropical Medicine 8 (2015) 104–111.

[34]. N. Khalaf, K. Ashok, A. Shakya, Antioxidant activity of some common plants, Turkish Journal of Biology 32 (2008) 51-55.

[35]. A.G. Agbor, J. Vinson, E.J. Oben, Ngongang, J.Y. Comparative analysis of the in vitro antioxidant activity of white and black pepper, Nutrition Research 26 (2006) 659-663.

[36]. M.G. Pontifex, M.M.A.H. Malik, E. Connell, M. Müller, D. Vauzour, Citrus polyphenols in brain health and disease: current perspectives, Frontiers in Neuroscience 15 (2021) 640648.

Received: 19.11.2022
Received in revised form: 12.09.2023
Accepted: 13.09.2023