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Chemical Composition, Antioxidant Activity and Nutritional Potential of Two Species of Okra: *Abelmoschus caillei* and *Abemonchus moschatus*

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Abstract This work aims to study the intervarietal variation of chemical and biochemical characterization and antioxidant capacity in fresh immature fruits of four varieties of *Abelmoschus caillei* and one variety on *Abelmoschus moschatus*. The phytochemical screening of fresh immature fruits powder was estimated using the experimental methodology of Houghton et al. [1]. Total phenolic content was determined by the method of Folin-Ciocalteu while total flavonoid and condensed tannins content were estimated using the $AlCl_3$ method and vanillin method respectively. The antioxidant capacity in the forms of DPPH (2, 2-diphenyl-1-picrylhydrazyl) and ferric reducing/antioxidant power (FRAP) analysis was evaluated by spectrophotometric methods. Protein and sugar contents were evaluated by the method of Gornall [2] and Dubois [3]. Atomic absorption spectrophotometry was used for mineralogic analyses.

The phytochemical screening revealed in fresh immature fruits of *A. esculentus* the presence of catechin tannins, mucilages, flavonoids, leuco-anthocyanins, reducing compounds, sterols and terpenes. Variety V_{61} seems to be the richest in total polyphenols (28.655 mg EAG/100mg of dry matter) and in flavonoïds (71.638 mg CQ/g of dry matter). However, condensed tannins contents are substantially low. Variety V_{58} of *A. caillei* had the highest content of condensed tannins (13.602 ± 0.220 mg EC/g of dry matter). The highest protein content is observed in the variety V_{61} (21.546 ± 0.017 g/100 g). The zinc and iron contents are relatively low. Varieties V_{58} (58 mg/kg) and V_{55} (36 mg/kg) showed the high contents of zinc and iron. The copper content varied from 10 mg/Kg to 12 mg/kg. At 1mg/ml, the inhibition percentage of DPPH radical scavenging activity ranged from79.81 to 99.26%. The variety V_{61} had the highest DPPH scavenging capacity. The highest ferric ion reduction capacity was observed with the variety V_{55} (57.91 mmol EAA / g). Therefore, all varieties of okra studied are a potential source of nutrients and antioxidants that can be used in pharmaceutical and food preparations.

Keywords Antioxidant Activity, *Abelmoschus caillei, Abemonchus moschatus*

Introduction

Okra is one of the most important and widely grown vegetable crops grown throughout the tropic and subtropics. It is a member of the family Malvaceae and genus Abelmoschus. Okra is a very popular tasty and gelatinous

vegetable. Tender green pods 3-5 days old are used as a vegetable, generally marketed in the fresh state but sometimes in canned form [4]. In dry areas, fruits are cut into slices, dried in the sun and stored for long periods [4]. Grown from seed, in tropical. Subtropical and mediterranean climatic zones. Several studies reported that Okra provides an important input of vitamins and mineral salts, protein, fiber [5-7]. Most of these studies have focused on *A. esculentus* and very little chemical and biochemical data exists on *A. caillei* and *A. moschatus*. Moreover, despite the fact that Benin produces 56 564 tonnes of okra per year [8], there have been no studies on the chemical and biochemical characterization of *A. caillei* and *A. moschatus* varieties produced in Benin. Knowing that the composition of plants and vegetables varies from one region to another, in the present research, the phytochemical composition and content of phenolic compounds, flavonoids and condensed tannin were evaluated on fresh immature fruits of four varieties from *A. caillei* and one variety from *A. moschatus*. Furthermore we studied the biochemical composition and evaluated the antioxidant activity of extracts hydroethanolic of these fresh immature fruits by 2,2-diiphenly- 1-picrylhydrazyl (DPPH) free radical scavenging assay and, ferric reducing/antioxidant power (FRAP) analysis.

Material and Methods

Plants Materials

The fresh immature fruits of four varieties of *Abelmoschus caillei* (V_{55}, V_{57}, V_{58} and V_{60}) and one variety of *Abelmoschus moschatus* (V_{61}) were obtained from Department of Botanic, University of Abomey-Calavi, Republic of Benin, washed properly with distilled water and dried under shade at room temperature. This fresh immature fruits were blended into powdered form and stored in sterile containers until analysis.

Chemical Characterization

Phytochemical Screening

Screening was a qualitative chemical analysis based on differential staining reactions and/or precipitation of the major chemical compounds groups contained in plants. The experimental methodology adopted in this study was that of Houghton *et al.* [1]. The targeted compound were alkaloids, phenolic compounds (catechin tannins, gallic tannins, flavonoids, anthocyanins, leucoanthocyanin), quinine derivatives, saponosides, triterpenoids, steroids, mucilages, coumarins, reducing compounds and anthracene derivatives.

Extraction

10 g of fresh immature fruit powder of each variety were extracted for 24 hours by maceration with 100 mL of ethanol-water (50:50) at room temperature under magnetic stirring. The extracts were filtered and the filtrate was concentrated by rotary vacuum evaporation at 40 ℃ until obtaining a solid residue.

Estimation of Total Phenolic, Total Flavonoid and condensed Tannins contents

Total phenolic contents

The total phenolic content (TPC) was determined by a Folin Ciocalteu assay [9] using gallic acid as the standard. The mixture of the sample solution (400µL), 2mL of Folin-Ciocalteu's reagents solution, and 1.6 mL $NaCO_3$ (7%) was vortexed. The mixture was allowed to stand for 2h at room temperature. The absorbance was measured at 765 nm against distilled water as a blank. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/g sample) through the calibration curve of gallic acid.

Total flavonoids contents

Total flavonoid content was determined using a colorimetric method described previously [10]. Briefly, a dose of 0.25 mL of extract or catechin standard solution was mixed with 1.25 mL of distilled water in a test tube, followed by 75µL of a 5% $NaNO_2$ solution. After 6 min, 150µL of a 10% $AlCl_3$, $6H_2O$ solution was added and allowed to stand for another 5 min before adding 0.5 mL of 1 M NaOH. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using a UV-Visible Spectrophotometer. The results were calculated and expressed as micrograms of quercetin equivalents (mg of QE/g sample) using the calibration curve quercetin. Linearity range of the calibration curve was 10 to 1000 µg/mL.

Condensed tannins contents

Analysis of condensed tannin content (CTC) was carried out according to the method reported by Agbangnan and *al.*, 2012. To 50 µL of the suitably diluted sample, 3 mL of a 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added. The mixture stood for 15 min, and the absorption was measured at 500 nm against methanol as a blank. The amount of condensed tannin was calculated and expressed as mg catechin equivalents (mg of CE/g sample) using the calibration curve of catechin. Linearity range of the calibration curve was 50 to 1000 µg/mL (r = 0.99).

Biochemical Characterization

The total proteins contents

The total protein content was determined by the biuret assay [2] using Beef albumin serum (BSA) as the standard. This method is one of the most commonly used to determine the total protein in a sample. It is based on the complexation of Cu^{2+} to functional groups in the protein's peptide bonds. The formation of a Cu^{2+}-protein complex requires two peptide bonds and produces a violet-colored chelate product which is measured by absorption spectroscopy at 540 nm.

1 g of the sample was dissolved in 100 mL of distilled water. The mixture is then centrifuged at 3000 rpm for 15 min. 2 ml of the Gornall reagent are added to 0.5 ml of the filtrate obtained after maceration. 0.5 mL of distilled water are again added to this mixture and the whole is homogenized and incubated for 30 min. The absorbance of the mixture is then read at 540 nm against the white (Gornall reagent + distilled water) using a spectrophotometer. The amount of protein contained in the immature fruits of each variety was determined by a calibration curve from a concentration range of a beef serum albumin solution (BSA).

Total sugars contents

Initially, each sample in the form of powder was slurried with distilled water using a solid-liquid ratio of 10% (0.1 mg/ml) and stirred at room temperature. Subsequently, the samples were centrifuged for 15 min at 4000 rpm. The supernatant fluid was filtered through Whatman filter paper and the filtrate was used for determining total Sugar by the respective procedure. Quantitative determinations of total soluble sugars were performed using a phenol-sulfuric acid colorimetric procedure based on the absorbance at 490 nm of an aromatic complex formed between phenol and the carbohydrate. The amount of sugar present determined by comparison with a calibration using a reference standard of glucose on a UV-Visible spectrophotometer.

Determination of mineral contents

Minerals content analysis was determined according to AOAC, (2000). Copper (Cu), iron (Fe), and zinc (Zn) concentrations were measured by atomic absorption spectrophotometer.

***In vitro* antioxidant potential**

Ferric-Reducing antioxidant power (FRAP)

Initially, 10 g of fresh immature fruit powder of each variety were extracted for 24 hours by maceration with 100 mL of ethanol-water (50:50) at room temperature under magnetic stirring. The extracts were filtered and the filtrates were concentrated by rotary vacuum evaporation at 40 ℃ until obtaining a solid residue. Reducing ability was performed using the method described by Amoussa *et al*., [11]. Briefly, 2 ml of extracts (100 µg/ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50°C for 20 min followed by addition of 2 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 2 ml from each of the mixture earlier mentioned was mixed with 2 ml of distilled water and 0.4 ml of 0.1% (w/v) fresh solution of ferric chloride. After 10 min of reaction, the absorbance were read at 700 nm. Ascorbic acid was used to produce the calibration curve (y = 0.028x-0.024; R2 = 0.995). The iron (III) reducing activity determination was performed in triplicate and expressed in µMol Ascorbic Acid Equivalent (AAE)/g of extract.

DPPH Radical-Scavenging Activity

The ability of the extract scavenge of the 2,2-diphenyl-1-picrylhydrazyl radical was evaluated. The antioxidant activity was determined according to the method previously described [12]. Briefly, 1.5 ml of a freshly prepared methanolic solution of DPPH (2%) was mixed with 0.75 ml of extract solution (1 0.007 mg/ml). After 15 min of incubation in the dark, at room temperature, absorbencies were read at 517 nm against a blank sample consisting

of a 1.5 ml of methanol and 0.75 ml of extract solution. All tests were performed in triplicate. DPPH radical inhibition percentage was calculated according to the following formula:

$$\text{Inhibition (\%)} = [(AB - As)/ AB] \times 100$$

where, As is the sample (tested extract solution) absorbance and AB is the blank absorbance.

Statistical analysis

Data were presented as mean ± SD. The graphical representation of the data was performed using the Microsoft Excel 2007. The difference was considered statistically significant when the $p < 0.05$.

Results and Discussion

Chemical Characterization

Phytochemical screening

Table 1: Main Metabolites of fresh immature fruit powder of twelve varieties of *A. caillei* and *A. moschatus*.

Secondary metabolites		V_{55}	V_{57}	V_{58}	V_{60}	V_{61}
Reducing compound			+	+	+	+
Tannins	Cathechics	+	+	+	+	+
	Gallics	-	-	-	-	-
Sterols and terpenes		+	+	+	+	+
Flavonoids		+	+	+	+	+
Leuco-anthocyans		+	+	+	+	+
Mucilages		+	+	+	+	+
Alkaloïds		-	-	-	-	-
Coumarins		-	-	-	-	-
Saponins		-	-	-	-	-
Anthocyans		-	-	-	-	-
free anthraquinones		-	-	-	-	-
Combined	O-hétérosides	-	-	-	-	-
anthraquinones	C-hétérosides	-	-	-	-	-
Cyanogenic derivatives		-	-	-	-	-
Quinone derivatives		-	-	-	-	-

-: absence; +: presence; V: variety

The powders of fresh immature fruits of these varieties of okra were subjected to preliminary phytochemical analysis so as to find out the phytoconstituents present in the samples. Table I shows the different metabolites identified in the okra varieties studied. Various secondary metabolites have been identified in the fresh immature fruit of these varieties by a series of coloring and precipitation reactions more or less specific to each class of active ingredients. Among these secondary metabolites we have catechin tannins, mucilages, flavonoids, leuco-anthocyanins, reducing compounds, sterols and terpenes. However, the fresh immature fruits of these varieties do not contain Alkaloïds, Coumarins, saponins, Anthocyans, free anthraquinones, combined anthraquinones, Cyanogenic derivatives, and Quinone derivatives.

Total phenolic, flavonoid and condensed tannins of fresh immature fruits' Hydroethanolic Extracts.

Table 2: Total phenolics, flavonoids and condensed tannins content in fresh immature fruits of *A. esculentus and A. moschatus* varieties.

Varieties	TPT (mg/100mg)	TFT (mg/g)	TTC (mg/g)
V_{55}	27.182 ± 0.002^{m}	67.955 ± 0.007^{m}	7.50 ± 0.147^{m}
V_{57}	25.178 ± 0.005^{n}	62.946 ± 0.013^{n}	5.955 ± 0.073^{h}
V_{58}	25.210 ± 0.015^{o}	63.025 ± 0.039^{o}	13.602 ± 0.220^{o}
V_{60}	25.828 ± 0.002^{p}	64.568 ± 0.006^{p}	0.919 ± 0.036^{p}
V_{61}	28.655 ± 0.005^{q}	71.638 ± 0.013^{q}	11.25 ± 0.073^{q}

Means not followed by the same superscript letters in the same column are significantly different ($P < 0.05$).
Data are expressed as mean ± SE of replicate determinations (n = 2).
V stands for variety.

The total phenolic compounds, flavonoïds and condensed tanins of hydroethanolic extracts of fresh immature fruits of these varieties of Okra expressed respectively in mg of gallic acid equivalent per gram (mg GAE /100 mg), mg of quercetin equivalent per gram (mg EQ/g) and mg of catechin equivalent per g (mg GCE/g) of dry matter (MS) are indicated by the table 3.

Table 3: Total protein an utilizable carbohydrate content of the varieties from okra (dry weight bases).

Varieties	Protein total (g / 100 g)	Utilizable carbohydrate (g /100 g)
V_{55}	12.443 ± 0.008^{m}	3.6207 ± 0.070^{m}
V_{57}	9.216 ± 0.017^{n}	4.4421 ± 0.007^{n}
V_{58}	21.197 ± 0.008^{o}	7.7941 ± 0.014^{o}
V_{60}	5.878 ± 0.119^{p}	5.8869 ± 0.007^{p}
V_{61}	21.546 ± 0.0170^{q}	4.7899 ± 0.007^{q}

Means not followed by the same superscript letters in the same column are significantly different ($P < 0.05$).
Data are expressed as mean ± SE of replicate determinations (n = 2).
V stands for variety.

Total phenolic contents

The total phenolic contents among the extracts was determined using the standard curve equations (y= 1.0033x +0.0586; $R^2 = 0.993$). The amount of total phenolics measured by Folin-Ciocalteu method dependent ranged from 25.178 ± 0.005 to 28.655 ± 0.005^{q} mg GAE/ 100 mg MS. The highest content of total phenolics was detected in variety V_{61} of *A. moschatus* with 28.655 ± 0.005^{q} mg GAE/ 100 mg MS followed by the variety V_{55} (27.182 ± 0.002 mg GAE/ 100 mg). The lowest total phenolics content were obtained with the variety V_{57} (25.178 ± 0.005 mg GAE/100 mg MS).

Total flavonoid contents

The estimation of total flavonoids in the hydroethanilic extracts of fresh immature fruits of these varieties of okra was showed in table 2. The total flavonoid content among the various extracts was determined using standard curve equations (y=2.5177x +0.0437; R2 = 0.999). The total flavonoid content in hydroethanolic extracts of fresh immature fruits showed different results ranging from 62.946 ± 0.013 to 71.638 ± 0.013 mg QE/g MS. The variety V_{61} had the highest total flavonoid content (71.638 ± 0.013 mg QE/g MS) followed by the variety V_{55} (67.955 ± 0.007 mg QE/g MS). However the variety V_{57} had the lowest one (62.946 ± 0.013 mg QE/g MS).

To*tal condensed tannins contents*

The total condensed tannins content among the extracts was determined using the standard curve equations (y= 1.0033x +0.0586; $R^2 = 0.993$). The amount of total phenolic measured by vanillin method dependent ranged from 0.919 ± 0.036 to 13.602 ± 0.220 mg CE/ g MS. The highest content of total phenolic was detected in variety V_{58} with 13.602 ± 0.220 mg CE/ g MS. The lowest total phenolic content were obtained with the variety V_{33} (5.955 ± 0.073 mg CE/ g MS).

Biochemical Characterization

Total protein content

The main functions of proteins are growth and replacement of lost tissues in the human body [7]. Nwofia et al. [13] reported that diet is nutritionally satisfactory, if it contains high caloric value and a sufficient amount of protein. Table 3 shows the crude protein contents of the twelve varieties of Okra fresh immature fruits used in the study. The protein content of the fresh immature fruits of these okra varieties was varied significantly ($P < 0.05$) from 5.878 ± 0.119 g/ 100g in V_{58} to 21.546 ± 0.0170 g/100 g in V_{61} on dry weight basis. No study is performed on the total protein content of the varieties of *A. caillei* and *A. moschatus*.

However, several studies reported the protein content of the varieties of *A. esculentus*. So the mean value of the varieties obtained in the study is higher than the values reported by Adetuyi et al., [6] (13.61–16.27 g/ 100g) and Nwachukwu et al., [14] (4.81g/ 100 g) on A. esculentus varieties. Ogungbenle and Omosola [15] is also reported

that the crude protein content of Okra fresh immature fruits is (23.4 g/100 g) which is higher than the fresh immature fruits of the varieties in the present study. These varieties of okra can be considered a high protein vegetable when compared with *Moringa oliefera* (4.2 g/100 g), Amarantus (6.1 g/100 g), *Gnetum africanum* (1.5 g/100 g), and Pterocarpus (2.0 g/100 g). Effiong et al. 2009 and Ali, 2010 have shown that any plant foods that provides about 12% of their calorific value from protein are considered good source of protein. The fresh immature fruits of all varieties of Okra of present study, meet this requirements except the Varieties V_{60} and V_{57}, and this implies that these Okra fresh immature fruits can serve as a good source of protein.

Utilizable carbohydrate content

The utilizable carbohydrate contents of the fresh immature fruits of these okra varieties were varied significantly ($P<0.05$) from one variety to other. Table 3 shows the utilizable carbohydrate contents of the twelve varieties of fresh immature fruits of okra used in the study. The utilizable carbohydrate content of fresh immature fruits of these okra varieties varied from 3.6207 ± 0.070 g/100 g to 7.7941 ± 0.014 g/100 g in varieties V_{55} and V_{58}, respectively. The utilizable carbohydrate content of variety V_{58} had higher (7.7941 ± 0.014g/100 g), whereas varieties V_{55} had the lowest (3.6207 ± 0.070 g/100 g). The mean value of the varieties obtained in the study is lower than the values reported by Gemede et al., [7] (36.66 to 50.97g/100 g).

Mineral composition

Table 2: Mineral concentrations of varieties from Okra (dry weight bases).

Variétés	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)
V_{55}	41	36	12
V_{57}	32	32	10
V_{58}	58	32	12
V_{60}	43	35	12
V_{61}	44	28	8

Minerals are inorganic elements, some of which are essential nutrients. The major minerals (Ca, K, Na and Mg) and essential trace elements (Fe, Cu, Zn and Mn) play very important roles in human metabolism [16]. Deficiencies of these minerals can lead to metabolic disorders and organ damage, leading to acute and chronic disease and ultimately death [17]. The mineral composition of okra's varieties is shown in Table 4.

Zinc is distributed widely in plant and animal tissues and occurs in all living cells. It functions as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase [18]. Zn dependent enzymes are involved in macronutrient metabolism and cell replication [19-20]. It is required for normal testicular development [21] and for functions of the taste buds. It is needed for tissue repair and wound healing, plays a vital role in protein synthesis and digestion, and is necessary for optimum insulin action as zinc is an integral constituent of insulin. It is an important constituent of plasma [22,23]. Zinc content in the fresh immature fruits of these varieties from okra is shown in Table 4. The content of Zinc varied between 32 mg/Kg in V_{57} and 58 mg/ kg in V_{58}. Zinc content of fresh immature fruits from V_{58} had higher (58 mg/ Kg) while variety V_{57} had the lowest (32 mg/ Kg) on dry weight basis. The values obtained in this study are higher than the values reported by Adetuyi et al., [6] (12.9 mg/kg –13.7 mg/ kg). However this values are similar than the values reported by Gemede et al., [7] (38.3 mg/ kg – 63.1 mg/kg).

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes c, c1, a1, etc [22]. Iron is an essential trace element for haemoglobin format on, normal functioning of central nervous system and in the oxidation of carbohydrates, protein, and fats. Table 4 shows Iron content of the five varieties from Okra used in the study. The contents of Iron varied from 28 mg/kg in V_{61} to 36 mg/ kg in V_{55}. The Iron content of Okra variety V_{55} had higher (36 mg/ kg) whereas the varieties V_{61} had the lowest (32 mg/100 g) on dry weight basis. The values obtained in this study were far higher than the value reported by Adetuya et al. [6] which is varied from 8; 7 mg/kg to 9.6 mg/ kg. However these values were far lower than the values reported by Gemede et al., [7] (183.0 mg/ kg to 366.8 mg/ kg). This indicates that fresh immature fruits of okra are a rich source of Iron.

Copper content of the varieties of Okra fresh immature fruit is shown in Table 4. In this study, the copper content is varied from 8 mg/ kg (V_{61}) to 12 mg/ kg (V_{55}, V_{58} and V_{60}). The copper content of varieties V_{55}, V_{58} and V_{60} had higher (12 mg/ kg) while the variety V_{61} had the lowest (8 mg/ kg) on dry weight basis. The values of this study is lower than the values reported by dos Santos et al. [24] which is varied from 0.7 mg/kg to 2.14 mg/ kg.

***In vitro* antioxidant activity**

Several methods are used to determine the antioxidant activity of plants. Thus, our study involved two methods to assess the antioxidant activity of fresh immature fruits of various varieties of *A. esculentus*, namely, ferric reducing/antioxidant power (FRAP) analysis and DPPH radical scavenging activity.

Reducing power of fresh immature fruits extraits from A. esculentus

Table 3: Antioxydant activity obtained using the FRAP method

Variétés	FRAP (mmol EAA g^{-1})
V_{55}	57.91
V_{57}	6.81
V_{58}	6.38
V_{60}	5.72
V_{61}	6.38
AA	10.74

AA: Ascorbic Acid

In this assay, the compounds with reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanides (Fe^{2+}), which then react with ferric chloride to form ferric ferrous complex that is greenish in color [25]. In the current study, Ferric Reducing Antioxidant Power (FRAP) of the extracts varied from 5.72 to 57.91 mmol AAE g^{-1} (table 5). All varieties except the variety V_{55} showed lower activity in comparison with the Ascorbic Acid (10.74 mmol AAE g^{-1}).

DPPH Radical Scavenging Activity

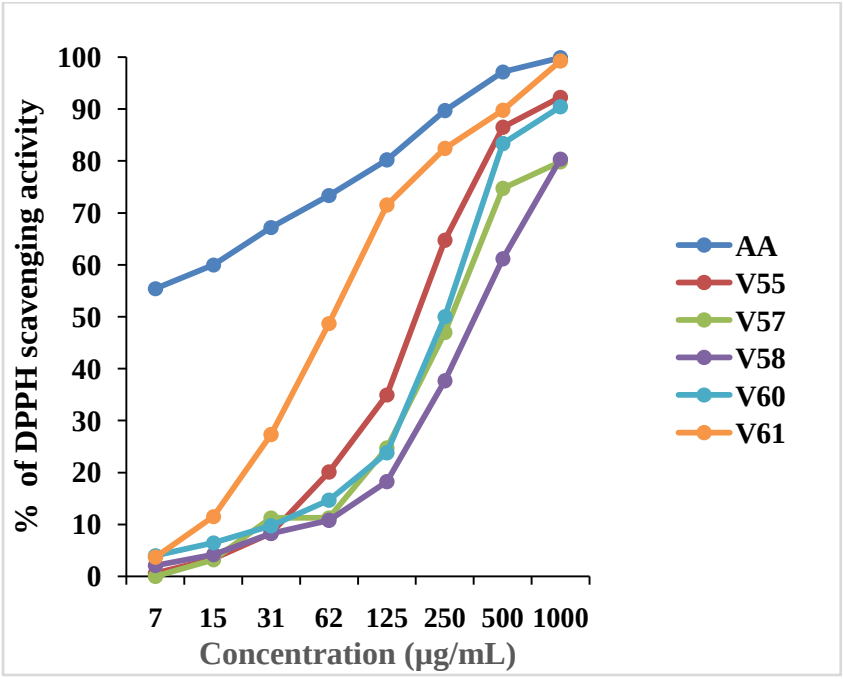

Figure 1: Radical scavenging activity of okra's varieties

The reduction of DPPH radical by antioxidants is evaluated by the decrease in absorbance at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation [26]. DPPH is usually used as a substance to evaluate the antioxidant potential of medicinal plants [27]. In this study, the DPPH

radical scavenging activities of extracts increased gradually in a dose concentration dependent manner (7.81-1000 µg/ml).The results show that at 1mg/ml the variation in antioxidant activities ranging from 79.81 to 99.26% (Fig.1). The results show that from 250 µg/ml to 1000 µg/ml, all varieties except the variety V_{58} showed significant activity (45 ≤IP%≤99.26) in comparison with the Ascorbic Acid (89.7≤IP%≤99.88) At 250 µg/ml, the varieties V_{55}, V_{60}, and V_{61} showed a percent inhibition greater than 50 %. At 500 µg/ml, the varieties V_{55} (IP% = 86.50), V_{60} (IP% = 83.33) and V= (IP% = 89.78) showed considerable activity compared with the control (Ascobic Acid, IP% = 97.2). At 125 µg/ml, all varieties showed an inhibition percentage (IP %) less than 50 % except the variety V_{60} (IP% = 71.50).

The fresh immature fruits are the healthy vegetable that is consumed in most areas of the world. Previous studies [28-29] have reported the wealth of fresh immature fruits of okra in phenolic compounds and their antioxidant effects. However, there were no studies regarding antioxidant potential of fresh immature fruit of different varieties of *A. esculentus* produced in Benin's republic. Several methods were used to determine the antioxidant activity of plants. In the current study, the results revealed that at the same concentration, the inhibitory percentage of DPPH radical was not the same. At each concentration, the variety V_{60} gave the highest percentage inhibition. These results showed that all extracts showed different percentages of inhibition of the DPPH scavenging activity on the concentration-dependent approach. Similar observations have been reported in previous studies [28-29].

Conclusion

The present work has revealed that the immature fresh fruits of the okra's varieties studied have proven to be a good potential source of vital nutrients such as phenolic compounds, crude protein, usable carbohydrates, zinc, copper and iron. Indeed, the immature fresh fruits of varieties V_{61} and V_{58} contain significantly higher amounts of phenolic compounds, crude protein, sugars, iron, copper and zinc than all varieties and can be treated as a cure for malnutrition in developing countries. The variety V_{55} has a free radical reduction capacity greater than that of ascorbic acid.

References

[1]. Houghton P.J., Raman A. (1998). Laboratory handbook for the fractionation of natural extracts London: chapman et al.

[2]. Gornall, A. G., Bardawill C. J. and David, M. M. (1949). Determination of serum proteins by means of the biureto reaction. Journal of Biological Chemistry. 177, 751-766.

[3]. Dubois M. Gilles K. (1999). Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. *Biologia Plantarum*, (42), 2, 233-239.

[4]. Hamon, S. (1988). Organisation évolutive du genre Abdmoschus (gombo). ORSTOM, Paris. TDM NP4G. 191.

[5]. Saifullah M. and Rabbani MG: 2009. Evaluation and characterization of okra (*Abelmoschus esculentus* L. Moench.) genotypes. SAARC Journal of Agriculture 7: 92-99.

[6]. Adetuyi F. O., Osagie A. U. and Adekunle A. T. (2011). Nutrient, antinutrient, mineral and bioavailability of okra *Abelmoschus esculentus* (L) Moench. American Journal of Food and Nutrition. 1, 49-54.

[7]. Gemede H. F., Gulelat D. H., Fekadu B., Ashagrie Z. W. and Sudip K. R. (2016). Proximate, mineral, and antinutrient compositions of indigenous Okra (*Abelmoschus esculentus*): implications for mineral bioavailability. Food Science and Nutrition. 42, 223–233.

[8]. MAEP. (2011). Centre régional pour la promotion agricole de l'Atacora-Donga : plan régional de développement de la filière cultures maraichères 13-14.

[9]. Singleton, V. I., Lamuela-Raventos, R. M. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Method in Enzymology. 2012; 299, 15.

[10]. Xu B.J., Chang S.K.C.A., comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents, Journal of Food Science. 2007; 72(2): 160-161.

[11]. Amoussa Abdou Madjid O., Ambaliou Sanni and Latifou Lagnika (2015). Antioxidant activity and total phenolic, flavonoid and flavonol contents of the bark extracts of *Acacia ataxacantha*. Journal of Pharmacognosy and Phytochemistry; 4(2): 172-178.
[12]. Velazquez E, Tournier HA, Mordujovich de Buschiazzo P, Saavedra G, Schinella GR. Antioxydant activity of Paraguayan plant extracts. Fitoterapia. 2003; 74, 91-97.
[13]. Nwofia, G. E., N. Nwogu Victoria, and B. K. Nwofia. 2012. Nutritional variation in fruits and seeds of pumpkins (Cucurbita Spp) accessions from Nigeria. Pak. J. Nutr. 11:946–956.
[14]. Nwachukwu, E. C., R. Nulit, and R. Go. 2014. Nutritional and biochemical properties of Malaysian okra variety. Adv. Med. Plant Res. 2:16–19.
[15]. Ogungbenle, H. N., and S. M. Omosola. 2015. The comparative assessment of nutritive values of dry nigerian okra (*Abelmoschus esculentus*) fruit and oil. Int. J. Food Sci. Nutr. Eng. 5:8–14.
[16]. Gorinstein S., Z. Zachwieja, M. Folta, H. Barton, J. Piotrowicz, M. Zemser, M. Weisz, S. Trakhtenberg, M.O. Belloso, Comparative contents of dietary fiber, total phenolics, and minerals in persimmons and apples, J. Agric. Food Chem. 49 (2001) 952–957.
[17]. Ozden O., N. Erkan, S. Ulusoy, Determination of mineral composition in three commercial fish species (Soleasolea, Mullussurmuletus, and Merlangiusmerlangus), Environ. Monit. Assess. 170 (2010) 353–363.
[18]. Soetan, K. O., Olaiya C. O., and Oyewole O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: a review. African journal of food sciences. 4, 200–222.
[19]. Arinola O. G. (2008). Essential trace elements and metal binding proteins in Nigerian consumers of alcoholic beverages. Pak. J. Nutr. 7(6): 763-765.
[20]. Hays VW, Swenson MJ (1985). Minerals and Bones. In: Dukes' Physiology of Domestic Animals, Tenth Edition pp. 449-466.
[21]. Merck V. M. (1986). The Merck Veterinary Manual. Sixth Edition. A handbook of diagnosis, therapy and disease prevention and control for the veterinarian. Published by Merck and Co., Inc., Rahway, New Jersey, USA.
[22]. Malhotra V. K. (1998). Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India
[23]. Murray R. K., Granner D.K., Mayes P. A., Rodwell V. W. (2000). Harper's Biochemistry, 25 th Edition, McGraw-Hill, Health Profession Division, USA.
[24]. dos Santos Ivanice F. , Ana dos Santos M. P., Uenderson Barbosa A., Jeane Lima S., Débora dos Santos C., Geraldo Matos D. (2013). Multivariate analysis of the mineral content of raw and cooked okra (*Abelmoschus esculentus* L.). Microchemical Journal. 110, 439–443.
[25]. Jothy S. L., Aziz A., Chen Y. Sasidharan S. (2012). Antioxidant activity and hepatoprotective potential of Polyalthia longifolia and Cassia spectabilis leaves against paracetamol-induced liver injury. Evidence-based complementary and alternative medicine; 2012:10.
[26]. Nalini R, Anuradha R. Phytochemical Screening and "In- Vitro" Antioxidant Activity of Ethanolic Flower Extracts of Punica granatum. Int J Pharm Sci Rev Res. 2015; 30, 353-360.
[27]. Shah R, Kathad H, Sheth R, Sheth N. *In vitro* antioxidant activity of roots of *Tephosia purpurea* Linn. Int. J Pharm. Sci. 2010; 3, 30-33.
[28]. Lianmei Hu, Wenlan Yu, Ying Li, Nagendra Prasad, and Zhaoxin Tang. Antioxidant Activity of Extract and Its Major Constituents from Okra Seed on Rat Hepatocytes Injured by Carbon Tetrachloride. BioMed Research International. 2014**;** 2014, 1- 9.
[29]. Fangbo Xia, Yu Zhong, Mengqiu Li, Qi Chang, Yonghong Liao, Xinmin Liu and Ruile Pan. Antioxidant and Anti-Fatigue Constituents of Okra. Nutrients. 2015; 7, 8846–8858..