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Phytochemical Composition and Antioxydant Capacity of *Abelmoschus esculentus* l. Fresh Immature Fruits

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Abstract This work aims to study the intervariation of chemical composition and antioxidant capacity of fresh immature fruits of 12 varieties of *Abelmoschus esculentus*. The phytochemical screening of fresh immature fruits powder of *A. esculentus* was realised using the experimental methodology of Houghton. Total phenolic content was determined by using the Folin-Ciocalteu method while total flavonoids and condensed tannins content were estimated using the $AlCl_3$ method and vanillin method respectively. The antioxidant capacities in the forms of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was evaluated by spectrophotometric method. The phytochemical screening made upon the powder of fresh immature fruits of *A. esculentus* revealed the presence of catechin tannins, mucilages, flavonoids, leuco-anthocyanins, reducing compounds, sterols and terpens. The results showed also that total phenolic compounds content, flavonoids and condensed tannins values were higher in V_{16} extract: 25.514 ± 0.005 mg GAE/100mg of dry matter, 63.786 ± 0.013 mg QE/g of dry matter and 12.242 ± 0.036 mg CE/g of dry matter. At 1 mg/mL, the inhibition percentage of DPPH radical scavenging activity ranged from 60.40 to 92.71%. The variety V_{16} had the highest DPPH scavenging capacity. Hence, the variety V_{16} represents a potential source of phenolic compounds and antioxidants and could be used in pharmaceutical and food preparations.

Keywords: *phytochemical screening, Abelmoschus esculentus, antioxidant capacity, intervariation*

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1. Introduction

Vegetables are important sources of macronutrients and micronutrients and have played an important role in the traditional diets of many regions throughout the world. In addition to their nutritional value, it has long been recognized that vegetables are functional foods that both promote good health and have therapeutic properties [1]. Many researchers indicate that vegetables may serve as an excellent dietary source of natural antioxidants for disease prevention and health promotion [2]. This potential is linked to their richness in secondary metabolites, among which are the phenolic compounds. Polyphenols are present in high amounts in most of foods plant and beverages [3] which cannot be synthesized by humans [4]. In the 1990s, several epidemiological studies demonstrated that dietary polyphenol consumption is associated with a reduced risk of cardiovascular disease [5,6]. As the basic and clinical research progressed, multiple functions of polyphenols contributing to human health were identified [7,8]. To help the indigenous population to fight against many diseases, it is necessary to identify and propose to them the plants food rich in phenolic compounds and accessible at a lower cost.

Okra, *Abelmoschus esculentus* (L.), is an important vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy season [9,10]. It is widely grown in Africa, Asia, Southern Europe and America [11]. Millions of tons have been grown in India (3.5 million tons), Nigeria (0.73 million tons), Pakistan (0.12 million tons), Ghana (0.10 million tons), Egypt (0.08 million tons) and Benin (56 564 tons) [9,12]. Okra is a multipurpose crop due to its various uses of the pods, fresh leaves, buds, flowers, stems, and seeds. Okra immature fruits, which are consumed as vegetables, can be used in salads, soups, and stews, fresh or dried, fried or boiled [13]. In addition, the plant has been used medicinally in treatment of several disorders. Anti-cancer, antimicrobial and hypoglycemic activities of plant are reported. The anti-ulcer activity of fresh fruits is recently reported [14].

But most of these studies were performed on the leaves, roots and the seeds of *A. esculentus*. Very few of scientific studies are focused on fruits whereas it is the most consumed part of the plant. In addition, no study is performed on the phytochemistry and antioxydant activity of the varieties of okra produced in Benin's Republic to our knowlegde. Hence, this work aims to study the intervariation of chemical composition and antioxidant capacity in fresh immature fruits of 12 varieties of *Abelmoschus esculentus*.

2. Material and Methods

2.1. Plants Materials

Plants Materials: The fresh immature fruits of twelve varieties of Okra (*Abelmoschus esculentus*) 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 flasks until analysis.

2.2. Phytochemical Screening

Screening is a qualitative chemical analysis based on differential staining and/or precipitation reactions of the major chemical compounds groups contained in plants. The experimental methodology adopted in this study was that of [15]. 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.

2.3. Extraction

10 g of fresh immature fruit powder of each variety of *Abelmoschus esculentus* 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.

2.4. Estimation of Total Phenolic Compounds, Total Flavonoids and Condensed Tannins Contents

2.4.1. Total Phenolic Contents

The total phenolic compounds content (TPC) was determined by a Folin Ciocalteu assay [16] using gallic acid as the standard. The mixture of the sample solution (400 µL), 2 mL of Folin-Ciocalteu's reagents solution, and 1.6 mL Na_2CO_3 (7%) was vortexed. The mixture was allowed to stand for 2 h 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 dry matter) through the calibration curve of gallic acid.

2.4.2. Total Flavonoids Contents

Total flavonoid content was determined using a colorimetric method described previously [2]. 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 adding 75µL of a 5% $NaNO_2$ solution. After 6 min, 150 µL of a 10% $AlCl_3$ 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 dry matter) using the calibration curve quercetin. Linearity range of the calibration curve was 10 to 1000 µg/mL.

2.4.3. Condensed tannins contents

Analysis of condensed tannin content was carried out according to the method of [17]. 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 dry matter) using the calibration curve of catechin. Linearity range of the calibration curve was 50 to 1000 µg/mL (r = 0.99).

2.5. *In vitro* Antioxidant Potential DPPH Radical-Scavenging Activity

The ability of the extract scavenge of the 2,2-diphenyl-1-picrylhydrazyl radical was evaluated. In the presence of antioxidant which is typical for DPPH free radical decays, the change in absorbency at 517 nm is followed spectrophotometrically. The antioxidant activity was determined according to the method previously described [18]. 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, absorbance was 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:

$$\text{Inhibition } (\%) = \left[(AB - As) / AB \right] x100 \text{ were}$$

AS: is the sample (tested extract solution) absorbance and AB: is the blank absorbance.

2.6. 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 $p < 0.05$.

3. Results and Discussion

3.1. Phytochemical Screening

The powders of fresh immature fruits of twelve varieties of *Abelmoschus esculentus* were subjected to preliminary phytochemical analysis so as to find out the phytoconstituents present in the samples. Table 1 shows the different metabolites identified in the plant materials studied. Various secondary metabolites have been identified in the fresh immature fruits of these varieties by a series of color and precipitation reactions more or less specific to each class of active ingredients. Among these secondary metabolites were 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.

3.2. Total Phenolic Compounds, Flavonoids and Condensed Tannins Contents of Hydroethanolic Extracts of *Abelmoschus* Fresh Immature Fruits.

The total phenolic compounds, flavonoïds and condensed tanins of hydroethanolic extracts of fresh immature fruits of these varieties of Okra expressed respectively out of equivalent mg of gallic acid per hundred milligrams (mg GAE /100mg) out of equivalent mg of quercetin per gram (mg QE/g) and out of equivalent mg catechin (mg CE /g) of dry matter (DM) are indicated by the Table 2.

3.2.1. Total Phenolic Compounds Contents

The total phenolic compounds contents among the extracts was determined using the standard curve equations (y= 1.0033x + 0.0586; R2 = 0.993). The amount of total phenolics measured by Folin-Ciocalteu method dependent ranged from 20.210 ± 0.005 to 25.514 ± 0.005 mg GAE/100 mg DM. The highest content of total phenolics was detected in variety V_{16} with 25.514 ± 0.005 mg GAE/100 mg DM followed respectively by the varieties V_3 (25.247 ± 0.010 mg GAE/100 mg) and V_{32} (25.016 ± 0.042 mg GAE/100 mg DM). The lowest total phenolics content were obtained with the variety V_6 (20.210 ± 0.005 mg GAE/100 mg DM).

3.2.2. Total Flavonoids Contents

The estimation of total flavonoids in the hydroethanolic extracts of fresh immature fruits of these varieties of okra was showed in Table 2. The total flavonoids content among the various extracts was determined using standard curve equations (y = 2.5177x + 0.0437; R2 = 0.999). The total flavonoids content in hydroethanolic extracts of fresh immature fruits showed different results ranging from 50.525 ± 0,013 to 63.786 ± 0.013 mg QE/g DM. The variety V_{16} had the highest total flavonoids content (63.786 ± 0.013 mg QE/g DM), however the variety V_6 had the lowest one (50.525 ± 0.013 mg QE/g DM).

Table 1. Main Metabolites of fresh immature fruits powder of twelve varieties of *Abelmoschus esculentus*

		Abelmoschus esculentus											
Secondary metabolites		**V_3**	**V_5**	**V_6**	**V_{16}**	**V_{19}**	**V_{23}**	**V_{32}**	**V_{33}**	**V_{37}**	**V_{41}**	**V_{42}**	**V_{52}**
Reducing compounds		+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Cathechic	+	+	+	+	+	+	+	+	+	+	+	+
	Gallic	-	-	-	-	-	-	-	-	-	-	-	-
Sterols and terpens		+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids		+	+	+	+	-	+	+	+	+	-	-	+
Leuco-anthocyans		+	+	+	+	+	+	+	+	+	+	+	+
Mucilages		+	+	+	+	+	+	+	+	+	+	+	+
Alkaloïds		-	-	-	-	-	-	-	-	-	-	-	-
Coumarins		-	-	-	-	-	-	-	-	-	-	-	-
Saponins		-	-	-	-	-	-	-	-	-	-	-	-
Anthocyans		-	-	-	-	-	-	-	-	-	-	-	-
free anthraquinones		-	-	-	-	-	-	-	-	-	-	-	-
Combined anthraquinones	O-hétérosides	-	-	-	-	-	-	-	-	-	-	-	-
	C-hétérosides	-	-	-	-	-	-	-	-	-	-	-	-
Cyanogenic derivatives		-	-	-	-	-	-	-	-	-	-	-	-
Quinone derivatives		-	-	-	-	-	-	-	-	-	-	-	-

-: absence; +: presence; V: variety.

Table 2. Total phenolic compounds, flavonoids and condensed tannins contents in fresh immature fruits of *Abelmoschus esculentus* varieties

Varieties	TPC (mg GAE/100 mg)	TFC (QE mg/g)	TTC (CE mg/g)
V_3	25.247 ± 0.010^a	63.117± 0.026^a	12.867 ± 0.073^a
V_5	24.556 ± 0.003^b	61.390 ± 0.006^b	9.779 ± 0.073^b
V_6	20.210 ± 0.005^c	50.525 ± 0.013^c	6.801 ± 0.036^c
V_{16}	25.514 ± 0.005^d	63.786 ± 0.013^d	12.242 ± 0.036^d
V_{19}	21.891 ± 0.005^e	54.726 ± 0.013^e	10.073 ± 0.073^e
V_{23}	22.888 ± 0.016^f	57.221 ± 0.039^f	10.882 ± 0.147^f
V_{32}	25.016 ± 0.042^g	62.539 ± 0.105^g	12.132 ± 0.367^g
V_{33}	22.318 ± 0.003^h	55.797 ± 0.006^h	5.955 ± 0.073^h
V_{37}	22.437 ± 0.005^i	56.092 ± 0.013^i	8.014 ± 0.073^i
V_{41}	20.599 ± 0.005^j	51.496 ± 0.013^j	8.75 ± 0.073^j
V_{42}	23.477 ± 0.005^k	58.692 ± 0.013^k	6.911 ± 0.147^k
V_{52}	22.447± 0.005^l	56.118 ± 0.013^l	9.485 ± 0.367^l

TPC: Total phenolic compounds content; TFC: Total flavonoids content; TTC: Total condensed tannins content.

3.2.3. Total Condensed Tannins Contents

The total condensed tannins content among the extracts was determined using the standard curve equations (y = 1.0033x + 0.0586; R2 = 0.993). The amount of total phenolic measured by vanillin method dependent ranged from 5.955 ± 0.073 to 12.867 ± 0.073 mg CE/g DM. The highest content of total phenolic was detected in variety V_3 with 12.867 ± 0.073 mg CE/g DM followed respectively by the varieties V_{33} (5.955 ± 0.073 mg CE/g DM), V_6 (6.801 ± 0.036 mg CE/g DM) and V_{42} (6.911 ± 0.147 mg CE/g DM). The lowest total condensed tannins content were obtained with the variety V_{33} (5.955 ± 0.073 mg CE/g DM).

3.5. In Vitro Antioxidant Activity DPPH Radical Scavenging Activity

The reduction of the DPPH radical by the antioxidants is evaluated by the decrease of the absorbance of the DPPH solution at 517 nm. This decrease is due to the reaction between antioxidant molecules and free radicals which results in the scavenging of the radical by hydrogen donation [19]. DPPH is usually used as a substance to evaluate the antioxidant potential of medicinal plants [20]. In this study, the DPPH radical scavenging activities of extracts increased gradually in a dose concentration dependent (7.81-1000 µg/mL).The results show that at 1mg/mL the variation in antioxidant activities ranging from 60.40 to 92.71% (Figure 1). The results show that from 250 µg/mL to 1000 µg/mL, all varieties except the varieties V_{33}, V_{37}, V_{41}, V_{42} and V_{52} showed significant activity ($45 \leq IP\ \% \leq 92.71$) in comparison with the vitamin C ($89.7 \leq IP\ \% \leq 99.88$) at 250 µg/mL. The varieties V_5, V_6, V_{16} and V_{23} showed a inhibition percentage greater than 55%. At 500 µg/mL, the varieties V_6 (IP% = 83.96) and V_{16} (IP % = 87.17) showed considerable activity compared with the control (Vitamin C. IP% = 97.2). From 7.81 to 125 µg/mL, all varieties showed an inhibition percentage (IP %) less than 50 %.

Immature fresh fruits are the most consumed vegetables in most parts of the world. Previous studies [21,22] 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. Thus, our study involved one method to assess the antioxidant activity of fresh immature fruits from okra, namely, DPPH scavenging activity analysis. Among the most widely used procedures for measurement of antioxidant activity capacity, the DPPH radical scavenging analysis is one of the best known, accurate, and frequently employed to measure the electron transfer ability of the plant extracts [23,24]. DPPH is a stable radical commonly used to determine the antioxidant activity of various compound. It is a stable free radical because of its spare electron delocalization over the whole molecule. 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_{16} 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 [21,22].

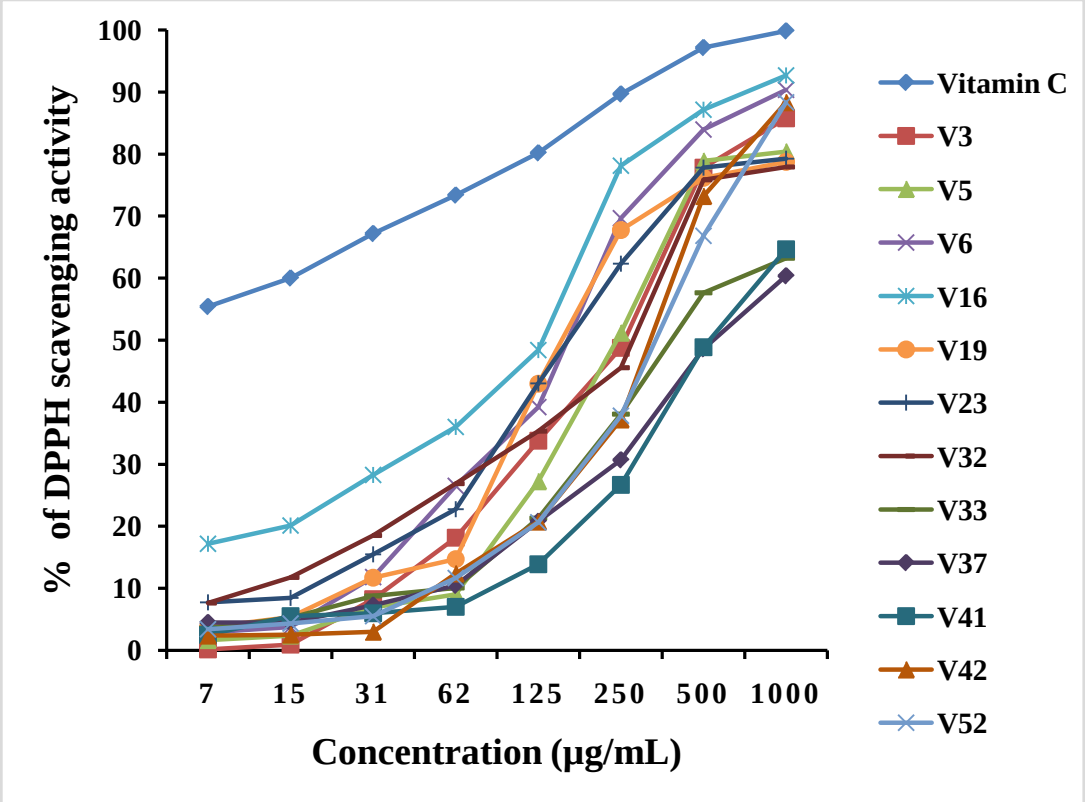

Figure 1. Radical scavenging activity of okra's varieties

4. Conclusion

In summary, the results of this study clearly showed significantly different phenolic contents and antioxidant activities in fresh immature fruits of extracted different varieties from okra. The richest out of all varieties in phenolic compounds was the variety V_{16}. In addition, this variety had showed the hightest DPPH scavenging activity. Hence, the variety V_{16} represents a source of phenolic compounds and potential antioxidants that could be recommanded in pharmaceutical and food preparations.

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