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Full Length Research Paper

Total flavonoid, phenolic contents and antioxidant scavenging activity in 25 accessions of okra (*Abelmoschus spp* L.)

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Twenty-five (25) accessions of Okra (*Abelmoschus spp* L.) were collected from eight geographic regions of Ghana and were evaluated for their phytochemical constituents. The objective of the study was to assess total flavonoid, phenolic and antioxidant activity in the accessions. Results indicate that, there are statistically significant differences (p≤0.05) in Total Flavonoid Contents (TFCs), Total Phenolic Contents (TPCs) and Total Antioxidant Contents (TAAs) recorded for both the ethanolic and aqueous extracts of the accessions, indicating genetic variability among them.The high variability observed in the amounts of TFCs, TPCs and TAAs in the fresh fruits of okra, makes okra a good source of natural antioxidants.

Keywords: Okra, *Abelmoschus*, phytochemicals, antioxidants, free radicals, health benefits, DPPH, phenolics, flavonoids.

INTRODUCTION

Free radicals are highly reactive chemical substances (Long et al., 2007) such as peroxide, hydroxyl radical and singleton oxygen that travel around in the body and cause damage to body cells (Alia et al., 2003). Free radical damage is one of the most prominent causes of devastating diseases that are responsible for killing millions of people in the world and this can manifest as heart attacks and cancers (Amic et al., 2003). Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes such as conversion of food into energy in the body (Oboh and Rocha, 2006).

Antioxidants are powerful free radical scavengers in the human body. Several researches on antioxidants in biological systems have confirmed their neutralising effects on oxidative stress that predispose the human body to lethal diseases and thus, generating keen interest in assessment of antioxidant potentials of consumable food compounds (Karadag et al., 2009). Antioxidants comprise a number of chemical compounds including flavonoids and phenolics.

Major sources of flavonoids and phenolics are vegetables and fruits (Cai et al., 2004). Current growing interest in antioxidants lies in their capability to preclude and avert deleterious effects of free radicals in the body and deterioration of fats and other food constituents (Apea-Bah et al., 2009). Okra has been described as a 'storehouse' of nutrients (Siemonsma and Kouame, 2004; Tidall, 1983). In spite of the health-benefit potential of the crop, there is a dearth of information on antioxidant-phyto constituents present in the vegetable.

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Table 1. Geographical collection sites (regions) of 25 accessions of Okra

Source	Number collected	Accession
Greater Accra region	8	Labadi, Asontem-GAR, Atomic, Cs-Legon, Legon fingers, Clemson spineless, Indiana, Volta
Ashanti region	6	Asontem-ASR, Agric type I, Debo, Asante type II, Kortebortor-ASR, Agric short fruit
Central region	1	Cape
Eastern region	1	Asontem-ER
Brong-Ahafo region	5	Yeji-Local, Asontem nv, Asontem-BAR, Kortebortor-BAR, Nkran Nkuruma
Western region	1	Juaboso
Upper East region	3	Mamolega, Wune mana, Mapelega

The relative contents of flavonoids and phenolics in fruits and vegetables can be determined using a number of methods including oxygen radical absorbance capacity (ORAC) assay (Huag et al., 2005; Ou et al., 2001), diphenylpicryl-hydrazyl (DPPH) assay (Botchway et al., 2007), ferric reducing/antioxidant power (FRAP) (Karadag et al., 2009), deoxyribose assay (Adom and Liu, 2005), quantification of products formed during peroxidation (Chumark et al., 2008), low-density lipoprotein (LDL) oxidation (Gheldoff and Engeseth, 2002) and total antioxidant parameter (TRAP) (Huag et al., 2005). However,the diphenylpicryl-hydrazyl (DPPH) method (Botchway et al., 2007) is commonly used as it is technically simple, rapid and inexpensive method to measure and determine antioxidant activity of foods, which employs a stable 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical.

A measure of flavonoids and phenolics as well as total antioxidant capacity in okra will help understand the functional properties of the vegetable. The objective of the study was to determine the total flavonoid and phenolic contents as well as total antioxidant scavenging activity in 25 accessions of okra (*Abelmoschus spp* L.).

MATERIALS AND METHODS

Sample Preparation

Twenty-five (25) accessions of okra *Abelmoschus spp L.* (Table 1),assembled from eight geographical regions of Ghana,were planted on a 59m x 32m plot of land using the Randomised Complete Block Design with four replications at the research fields of the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission, between July, 2011 to January, 2012. Sample whole fruits were harvested at edible maturity stage (5-8 days after flowering) and lyophilised (freeze-dried) at the Ghana Research Reactor one (GHARR1) of the Ghana Atomic Energy Commission (GAEC) and subsequently homogenised in a stainless steel blender to obtain a powdery form. Two grammes (2g) of the powdered okra were poured into centrifuge tubes. 30ml of distilled water was added and stirred on a mechanical agitator for 2hours. This was topped up with 20ml of distilled water and agitated for additional 2hours, making a total volume of 50ml.The supernatant was then stored and kept in the freezer till needed.

Determination of total flavonoid content

Aluminium chloride colourimetric method was utilised for determination of flavonoids (Zhishen et al., 1999). 0.05ml of extract was mixed with 1.5ml of 99.9% ethanol (EtOH), 0.01ml of 1 M potassium acetate, 0.01ml of 10% aluminum chloride and 3.0ml of distilled water. The resulting mixtures were incubated for 30 minutes at room temperature and corresponding absorbance measured at 415 nm. All determinations were carried out in triplicates. A standard calibration curve was constructed using quercetin standard solutions of 0.0025mg/ml, 0.005mg/ml, 0.0075mg/ml, 0.001mg/ml and 0.012mg/ml.0.005ml of each standard was treated in the same manner as the samples above and a calibration linear regression equation of y = 0.043x was obtained, (where x = mg per Quercetin), R^2 = 0.973, where R is the coefficient of the regression line. Total flavonoid content (TFC) was expressed as mg of quercetin equivalents (QE) /g of extract according to the formula by Chang et al. (2002):

$$\text{Total Flavonoid Content} = \frac{(c \times df \times v)}{w}$$

where; c = concentration obtained from the standard curve; df = dilution factor; v = volume of stock solution; w = weight of okra extract used in the experiment. Spectrophotometric readings were evaluated for statistical significance using one-way analysis of variance (ANOVA) and means separated by the Duncan's multiple range tests (Statgraphics Centurion XVI, version 16.1.11,

USA) expressed as the Mean ± SD (standard deviation of the mean) upon three independent analyses. A p-value of 0.05 or less was considered as statistically significant.

Determination of total phenolic content

Total phenolic content (TPC) of the 25 accessions of okra was determined according to the Folin-Ciocalteau method (Kujala et al., 2000; Singleton et al., 1999), using gallic acid as a standard. Five grammes (5g) of the samples were dissolved in 5 ml of distilled water (50:50, v/v). 0.005ml of the extract was mixed with 3.0ml of distilled water (dH_2O) and 0.025ml of Folin-Ciocalteau reagents (FCR). The mixtures were allowed to stand for 5 minutes, and then 0.075ml of 20% Na_2CO_3 was added. After incubating the resultant reaction mixtures for 30 minutes at room temperature, absorbance values were measured spectrophotometrically at 760nm using a UV-VIS Spectrophotometer (Shimadzu Corporation, 1201, Kyoto, Japan). All determinations were carried out in triplicate. A calibration curve was derived using the following dilution regimes; 0.02mg/ml, 0.04mg/ml, 0.06mg/ml, 0.08mg/ml and 0.01mg/ml from a stock solution of 10mg/ml Gallic acid dissolved in water. 5.0ml each of these solutions were treated in same manner as the samples and a calibration linear regression equation established as y = 1.094x-0038, (where x is µg/g GAE), R^2 = 0.995, where R is the coefficient of the regression line, to explain the model. The total phenolic content in each extract was expressed as Garlic Acid Equivalent (GAE) in mg per gramme sample using the formula below:

$$\text{Total Phenolic Content} = \frac{c \times v}{m}$$

Where c = the concentration of gallic acid established from the calibration curve in mg/g;
v = the volume of okra extract in micro litres;
m = the weight of okra extract in grammes.

Determination of total antioxidant activity

Total antioxidant activity (TAA) in okra extracts was determined using the 1,1-diphenyl 1-2-picrylhydrazl (DPPH) method by Blois, (1958) and Botchway et al. (2007) with slight modification. A solution of 0.004%µM DPPH was prepared through dissolution of 0.004g of DPPH in 100ml methanol. 200µl of the extract was added to 3.8ml of 0.004% DPPH. Concentrations of 0.2, 0.1, 0.05, 0.025, 0.020 and 0.01 mg/ml of Gallic Acid were used to plot the standard curve. The reduction or inhibition ability of DPPH radicals was determined by the decrease in its absorbance at 517nm induced by antioxidants after thirty (30) minutes incubation in the dark. Methanol was employed as a blank and absorbance read three times for each sample. The activity of the test samples was determined as a reduction of the DPPH, which is also referred to as inhibition or quenching and defined mathematically by Hatano *et al*. (1988) as: $\%\ \text{Inhibition} = \frac{c-s}{c} \times 100$, where s is the sample absorbance and c is the absorbance of the blank. Scatter diagrams were plotted and linear regression computed as $y = ax+b$, where y is the percent inhibition and x is the concentration in mg/ml.

RESULTS AND DISCUSSION

Geographical collection sites

Table 1 displays the identities of the 25 accessions and the geographical collection sites in Ghana. The largest collection of eight accessions was made in the Greater Accra region. This is to be expected as the region has a large number of farmers and farmers' associations engaged in urban and peri-urban vegetable production for export or sale locally.

Total flavonoid content

Table 2 shows the total flavonoid contents (TFCs) of the 25 accessions of okra used in the study. The highest TFC (5159.21±12.90 mg/g/QE), was recorded by Agric Short Fruit while Cs-Legon registered the lowest TFC of 871.57±3.84 mg/g/QE in the ethanolic extract. However, in the aqueous extract, Cs-Legon recorded the highest TFC (2003.69 ±2.55), with Yeji-Local recording the least (122.48 ±2.69 mg/g/QE). Mean TFCs were 2288.20±27.75 mg/g/QE and 686.17±6.17 mg/g/QE for the ethanolic and aqueous extracts, respectively.

TFCs in the ethanolic extracts were higher than in the aqueous extracts, indicating that the ethanolic extraction method was more efficient than the aqueous extraction method. This also indicates that, flavonoids in pods of okra are more soluble in organic solvents such as ethanol than in water. Khomsug et al. (2010) obtained TFCs of 1075±0.02mg/g and 1424.8±0.02mg/g for pulped okra and okra seeds, respectively, while Adelakun et al. (2009) obtained 32.54±32.42mg/g for blanched okra seeds, 48.3±0.00mg/g for raw okra seeds, and 51.28mg/g for soaked okra seeds. Both authors concluded that okra contains insignificant amount of flavonoids. The current findings however indicate significantly higher contents of flavonoids in both ethanolic and aqueous extracts of entire pods (including seeds) of okra. This therefore contradicts the findings of the earlier workers.

Total Phenolic Content

The total phenolic contents (TPCs) of the 25 accessions

Table 2. Total Flavonoids and Phenolics in mature fruits of 25 accessions of okra

	Flavonoids		Phenolics	
Accession	**Concentration (ethanolic extract)**	**Concentration (aqueous extract)**	**Concentration (ethanolic extract)**	**Concentration (aqueous extract)**
Asontem-GAR	2264.98±0.00^{gh}	492.89±9.56^{k}	11.38±1.77^{defghi}	20.94±0.00^{fg}
Asontem-ASR	2338.87±8.31^{gh}	493.08±14.74^{k}	13.18±2.30^{cdef}	22.46±0.64^{ef}
Asante Type II	1339.72±2.12^{n}	465.12±2.16^{l}	10.21±1.84^{efghi}	19.66±1.39^{g}
Asontem-ER	2235.14±0.89^{hi}	330.21±0.94^{o}	10.63±1.20^{efghi}	19.76±0.87^{g}
Wune Mana	4980.50±1.23^{b}	1449.24±1.28^{c}	18.19±2.25^{b}	8.58±0.84^{l}
Labadi	1958.80±0.00^{ik}	379.29±0.96^{n}	9.02±0.28^{ghi}	19.74±0.11^{g}
Agric Type I	2420.54±3.36^{g}	345.52±1.89^{o}	12.317±2.21^{cdefg}	28.21±1.92^{d}
Clemson Spineless	1288.06±92.91^{n}	532.77±2.11^{j}	9.69±1.17^{fghi}	27.08±2.00^{d}
Volta	3204.58±2.34^{e}	400.22±2.16^{m}	14.59±2.54^{cd}	**6.82±0.09^{l}**
Agric Short Fruit	**5159.21±12.90^{a}**	487.67±14.92^{k}	24.12±4.64^{a}	39.46±0.15^{b}
Debo'	3387.60±499.67^{d}	1402.50±2.38^{d}	**25.83±5.30^{a}**	29.07±0.00^{d}
Juaboso	2086.82±1.55^{ij}	648.16±3.00^{g}	11.79±1.47^{defgh}	20.16±1.93^{fg}
Kortebortor-BAR	1515.50±3.88^{n}	279.72±33.92^{p}	15.76±1.71^{bc}	23.62±1.33^{e}
Legon Fingers	3601.42±0.65^{c}	693.07±3.84^{f}	9.89±0.61^{efghi}	16.92±0.61^{hi}
Indiana	1609.01±1.45^{mn}	750.83±28.82^{e}	10.51±0.63^{efghi}	19.97±0.00^{fg}
Asontem-BAR	1517.55±0.60^{n}	485.24±3.39^{k}	8.55±1.01^{hi}	16.38±0.00^{ij}
Mamolega	1181.91±0.45^{n}	517.91±2.51^{j}	12.23±1.26^{cdefg}	14.25±1.17^{jk}
Nkran Nkuruma	1940.77±15.19^{jkl}	480.62±3.88^{kl}	8.89±0.54^{ghi}	16.54±0.36^{ij}
Atomic	988.53±2.15^{o}	582.50±1.92^{i}	13.42±2.22^{cde}	19.56±0.24^{gh}
Cape'	3077.18±11.14^{ef}	179.98±4.04^{q}	10.18±3.27^{efghi}	12.99±1.22^{k}
Mapelega	1874.81±1.78^{kl}	1579.18±3.84^{b}	9.07±0.53^{ghi}	32.27±5.40^{c}
Kortebortor-ASR	1774.33±22.10^{lm}	1443.88±0.00^{c}	**8.01±0.37^{l}**	**63.22±3.95^{a}**
Yeji-Local	1595.61±2.24^{n}	**122.48±2.69^{r}**	18.26±3.03^{b}	13.37±0.75^{k}
Cs-Legon	**871.57±3.84^{o}**	**2003.69±2.55^{a}**	10.76±0.89^{efghi}	7.97±0.00^{l}
Asontem N V.	2993.19±2.93^{f}	608.39±6.68^{h}	18.35±0.79^{b}	8.33±0.00^{l}
MEAN	2288.20±27.75	686.17±6.17	12.99±1.75	21.09±0.99

±sd=standard deviation, mean with same letters in a column are not statistically different (p≥0.05) from each other according to Duncan's multiple range test. Values bolded and underlined refers to accession with the highest concentration; Bolded values represents accession with the lowest concentration. All concentrations were measured in mg/ml.

of okra used for the study are also shown in Table 2. In the aqueous extracts, the highest TPC was registered by Kortebortor-ASR (63.22±3.95mg/g/GAE) while Volta had the lowest TPC of 6.82±0.09mg/g/GAE. Similarly, Debo (25.83±5.30mg/g/GAE) and Kortebortor-ASR (8.0±0.37mg/g/GAE) had the highest and lowest TPCs in the ethanolic extracts, respectively.

Mean TPCs were 12.99±1.75 mg/g/GAE and 21.09±0.99mg/g/GAE for ethanolic and aqueous extracts, respectively. In spite of the comparatively low yields for both extracts, TPCs for all the accessions were generally higher in the aqueous extracts than in the ethanolic extracts, indicating that phenolic compounds in okra are more soluble in water than in organic solvents such as ethanol, and thus; corroborating the report of Owusu-Ansah (2010) who worked on moringa leaf.From the analysis of variance, statistically significant differences were observed among the 25 accessions of okra in terms of TPCs. This may be attributed to genetic differences among the type of accession.

The TPCs of the accessions of okra compares well with common fruits and vegetables noted for their relatively high phenolic constituents such as apple (29.63±0.64mg/g), banana (9.04±0.32mg/g), lemon (8.19±0.35mg/g), orange (8.12±0.11mg/g), pineapple (9.43±0.15mg/g), cranberry (52.72±2.15mg/g), strawberry (16.00±0.12mg/g), pear (7.06±0.16mg/g), and grape (4.96±0.26mg/g) (Weng et al., 2005; USDA, 1998).

Total antioxidant activity

Table 3 shows the concentration of each extract (ethanolic and aqueous) against their percentage inhibition of the DPPH radical scavenger for all 25 accessions of okra. The coefficient of regression (R^2) of the logarithmic trend line was 0.94, which implies that 94% of the variability in DPPH can be ascribed to the concentration of the extracts. The analysis of variance (ANOVA) showed that, concentration significantly

Table 3. Antioxidant Potential of 25 Accessions of Okra

	Ethanol Extract		Aqueous Extract	
Accession	**%Inhibition**	**Conc.(mg/ml)**	**%Inhibition**	**Conc.(mg/ml)**
Asontem-GAR	**16.74**	**506.49±0.00^{j}**	29.13	906.69±0.00^{efgh}
Asontem-ASR	18.53	665.02±0.13^{ij}	27.13	992.43±2.22^{etg}
Asante Type II	32.38	1012.00±1.11^{fgh}	22.06	678.33±0.00^{hijklmn}
Asontem-ER	21.68	732.7±1.22^{hij}	23.05	1074.27±821.62^{de}
Wune Mana	54.060	3030.38±69.31^{b}	48.47	2660.70±0.00^{b}
Labadi	31.97	941.63±1.05^{fghi}	17.94	514.14±0.00^{lmn}
Agric Type I	27.70	1183.44±0.76^{def}	17.43	726.92±1.52^{ghijklm}
Clemson Spineless	45.27	1885.32±732.38^{c}	21.65	877.83±0.00^{efghij}
Volta	32.14	1440.87±1.59^{de}	23.95	1060.73±1.59^{de}
Agric Short Fruit	46.24	3705.81±0.00^{a}	25.94	2039.89±0.00^{c}
Debo'	51.12	**3812.16±0.00^{a}**	50.84	**3791.25±2.61^{a}**
Juaboso	35.01	1206.49±0.61^{def}	23.47	796.16±1.22^{fghijk}
Kortebortor-BAR	46.12	2001.22±12.19^{c}	20.96	883.92±0.00^{efghi}
Legon Fingers	34.89	1001.85±1.02^{fgh}	21.51	605.54±2.03^{klmn}
Indiana	**55.97**	1829.58±483.00^{c}	24.90	795.55±0.00^{fghijk}
Asontem-BAR	44.80	1195.87±506.53^{def}	23.71	618.99±0.00^{ijklmn}
Mamolega	51.15	1483.52±0.00^{d}	**15.09**	**415.51±11.18^{n}**
Nkran Nkuruma	28.99	763.45±0.94^{hij}	18.18	467.97±0.94^{mn}
Atomic	23.16	942.22±13.17^{fghi}	23.84	971.48±2.93^{fg}
Cape'	25.56	1135.49±0.00^{efg}	14.27	612.19±4.77^{jklmn}
Mapelega	29.81	766.31±0.91^{hij}	30.43	782.77±0.00^{ghijkl}
Kortebortor-ASR	28.22	643.36±0.41^{ij}	**56.33**	1309.57±0.81^{d}
Yeji-Local	47.21	2050.14±0.15^{c}	16.60	690.34±1.52^{hijklm}
Cs-Legon	29.02	805.70±0.00^{ghij}	25.39	700.90±0.98^{hijklm}
Asontem N V.	22.89	705.49±1.66^{hij}	28.01	870.66±0.55^{efghijk}
MEAN	35.23	2891.44±32.34	26.01	1033.79±34.26

±sd=standard deviation, mean with same letters in a column are not statistically different (p≥0.05) from each other according to Duncan's multiple range test.Values bolded and underlined refers to accession with the highest concentration or inhibition percentage; Bolded values represents accession with the lowest concentration or inhibition percentage. Conc = concentration.

($p<0.05$) affected percent inhibition. Total mean percent inhibition of DPPH radical for ethanol extracts was 35.27% and 30.73% for the aqueous extracts of the accessions. Indiana registered the highest percent DPPH inhibition of 55.97% with a correspondent antioxidant activity of 1829.58±438.00mg/g, while Asontem-GAR recorded the lowest percent DPPH inhibition of 16.74% which corresponded to the least antioxidant activity of 506.49±0.00mg/g in the ethanol extract. Similarly, the accessions Kortebortor-ASR (56.33%, 1309.57±0.81mg/g) and Cape (14.27%, 612.19±4.77mg/g) had the highest and lowest percent DPPH inhibitions and antioxidant activities, respectively, in the aqueous extracts.

In general, the free radical scavenging potentials of extracts of the okra accessions were variable but dose- or concentration-dependent. However, the ethanol extracts of the accessions were better able to reduce or inhibit the free DPPH radicals than the aqueous extracts. This also suggests that compounds present in the okra accessions responsible for scavenging the free DPPH radicals have higher solubility in ethanol than in water.

These observations are consistent with results obtained by Chakrabortthy and Ghorpade (2010), Apea-Bah et al. (2009) and Wu et al. (2009) working with extracts of *Abutilon indicum* (L.), gambir (*Uncaria gambir*) and pigeon pea (*Cajanus cajan* (L.), respectively. The variable scavenging activities of the free DPPH radicals may be attributed to the variable flavonoid and phenolic contents in the extracts of the accessions (Maltas and Yildiz, 2012; Maltas et al., 2011). A number of authors have reported a strong correlation between antioxidant activities of plant extracts and their contents of phenolic compounds (Abdel-Hameed, 2009; Arcan and Yemenicioglu, 2009; Almela et al., 2006). There were, however, a few discrepancy observed between the ethanol and aqueous extracts, in that, not all accessions with high TFC(s) or TPC(s) necessarily gave a corresponding high scavenging activity.This corroborates earlier reports by Owusu-Ansah (2010) for moringa leaf,

Quartey (2010) for tomatoes and Prior et al. (2005) for dietary supplements.

CONCLUSIONS

Okra possesses high amounts of total flavonoids as well as moderate amounts of total phenolics, making it a good source of natural antioxidants. There is high variability among the 25 accessions with respect to TFCs, TPCs and total antioxidant activity. Agric Short Fruit and Cs-Legon recorded the highest (5159.21±12.90mg/g/QE) and lowest (871.57±3.84mg/g/QE) total flavonoid contents (TFC) in the ethanol extracts while Cs-Legon and Yeji-Local registered the highest (2003.69±2.55mg/g/QE) and lowest (122.48mg/g/QE) TFCs in the aqueous extracts, respectively. On the other hand, Kortebortor-ASR registered the highest total phenolic content of 63.22±3.95mg/g/GAE while Volta had the lowest TPC of 6.82±0.09mg/g/GAE in the aqueous extract. By contrast, Debo and Kortebortor-ASR recorded the highest (25.83±5.30mg/g/GAE) and lowest (8.0±0.37mg/g/GAE) TPCs in the ethanol extracts, respectively. Ethanol as an extraction solvent yielded higher mean total antioxidant activity than water. The accessions, Indiana (55.97%) and Kortebortor-ASR (56.33%) emerged as fore-runners with respect to total antioxidant activity following extraction in ethanol and water respectively.

Of the 25 accessions of okra investigated, none emerged with the highest contents of flavonoids, phenolics as well as exhibiting the highest potential of antioxidant activity. It may be desirable to pyramid all three characteristics into a composite variety through hybridisation.

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