PHENOLIC PROFILES AND ANTIOXIDANT ACTIVITIES OF ETHIOPIAN INDIGENOUS OKRA (*ABELMOSCHUS ESCULENTUS)* POD AND SEED ACCESSIONS: A NEW SOURCE OF NATURAL ANTIOXIDANTS

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Abstract
Eight pods and seeds of eight indigenous Ethiopian okra (Abelmoschus esculentus) accessions were evaluated for the first time, for their phenolic contents and antioxidant activities in order to find new sources of natural antioxidants. Antioxidant activities were evaluated by using DPPH scavenging, reducing power, metal chelating and ABTS scavenging assays. The results were compared with different synthetic antioxidants. The antioxidant levels of the pod and seed accessions increased with increasing concentration of the samples. Okra pod and seed accessions were a respective range of total phenol (mg GAE/g) 28.10-95.21 and 21.28-57.34; and total flavonoid (mg CE/g) 8.18-18.72 and 10.73-29.04. The EC_{50} values (mg/ml) of the pods and seeds of okra accession had respective ranges of DDPH scavenging 2.10-10.30 and 3.1->12; for reducing power were 1.20-4.20 and 1.18-4.30; for metal chelating were 0.50-1.52 and 0.32-1.11; for ABTS scavenging were 0.31-1.33 and 0.07-1.5. The study revealed that phenolic contents and antioxidant activities were varied widely across pod and seed accessions, indicating their promising potential as new sources of natural antioxidants and deserves more attention. Particularly, both okra pod and seed accession, OPA#6 is a potential source of natural antioxidants; that could be used in food preparations and industry by replacing synthetic antioxidants.

Keywords: Okra, antioxidant, phenolics, flavonoids, seed, pod, accessions.

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1. INTRODUCTION

Okra (*Abelmoschus esculentus L.*) is one of the important vegetable crops originated in Ethiopia (Kumar et al., 2013) and widely distributed widely in Africa, Asia, Southern Europe, and America (Alba et al., 2013). Okra is a multipurpose crop due to the various uses of its pods, fresh leaves, buds, flowers, stems and seeds (Gemede et al., 2015). Okra has been described as a 'storehouse' of nutrients (Ahiakpa et al., 2013). Okra is also known for being high in antioxidants activity in the different parts of the plant (Shui & Peng 2004). In addition, Arapitsas (2008) reported that okra seed was rich in phenolic compounds, mainly composed of flavonol derivatives and oligomeric catechins, suggesting that it might possess some antioxidant properties. Okra seed is rich source of natural phenolic (Huang et al., 2007; Arapitsas, 2008). According to Adetuyi & Ibrahim (2014), total phenolic content of the pulp and seeds of okra extracts were 10.75±0.02mg GAE/100g extract and 142.48±0.02 mg GAE/100g. Okra is also rich in flavonoid compounds that have antioxidant activity (Adelakun et al., 2009). Epidemiological studies have suggested that the consumption of foods rich in flavonoid compounds could reduce the risk of diabetes, cardiovascular diseases, obesity, hyperlipidemia, stroke and cancers (Verma et al., 2012). Therefore, promoting the consumption of important traditional vegetables such as okra could provide cheap sources of antioxidants, which is very important in human health. However, Hu et al. (2014) reported that there is little information on antioxidant properties of okra. In addition, even if okra is native to Ethiopia for the local Berta community, both food and medicinal value, it has been considered as underutilized

crop and there is no single published study available about antioxidant properties of this Ethiopian indigenous okra of different edible parts and accessions. Therefore, this study for the first time, examined phenolic contents and antioxidant activities of Ethiopian underutilized indigenous okra pod and seed accessions, inorder to find the possible new sources of natural antioxidants from edible okra. The study will strongly enhance the utilization of okra vegetable.

2. MATERIALS AND METHODS

2.1 Sample Collection

Okra accessions were collected from different agro-ecological locations in the regions by Assosa Agricultural Research Center in 2012 and 2013 harvesting seasons and planted on the research center plot under similar agronomic practice and management conditions during the 2014 main cropping season. The pods and seeds of eight okra accessions, namely OPA#1, OPA#2, OPA#3, OPA#4, OPA#5, OPA#6, OPA#7, and OPA#8 were collected from the center plots during the 2014 main okra harvesting season. The collected samples were separately coded and packed. Then it was kept in an ice box and transported to Center for research laboratory of Food Technology and Process Engineering Research in Wollega University, Ethiopia. Upon arrival in the laboratory, distilled water was used to wash each of the pod accessions. The washed pods were sliced by a stainless steel knife. Sun was used to dry the sliced okra pod accessions and then followed by oven drying at 45^{o}C. The seed accessions were manually removed from the pods, sorted and sun dried. Electric grinder was used to mill into fine powder until pass through 0.425 mm in diameter sieve size. To minimize heat build-up the fine powder samples were kept in airtight polyethylene plastic bags and stored in the desiccators until required for further analysis.

2.2 Sample extraction

Samples were extracted according to the procedures described by Woldegiorgis et al. (2014). Briefly, ten gram of each pod and seed accessions were extracted by stirring with 100 ml of methanol at 25^{0}C at 150 rpm for 24 h using temperature shaker incubator (MRWY-913F) and then filtered through Whatman No. 4 paper. Two additional 100 ml portions of methanol was used to extract the residue as described above. Dryness was used to evaporate the combined methanolic extracts at 40 ^{o}C using rotary evaporator (Mourdt R35834) and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4 ^{o}C for further use.

2.3. Antioxidant Activity

2.3.1 Determination of free radical scavenging activity

DPPH radical scavenging activity was determined according to procedures described by Woldegiorgis et al. (2014). Briefly, DPPH radical of 0.004% solution was prepared in methanol. Four milliliter of the solution was mixed with one milliliter of concentrations from 2 to 14 mg/ml of the extracts in methanol. Finally, the dark at room temperatures were used to incubate the samples for 30 min. By monitoring the decrease in absorbance at 517 nm, the scavenging capacity was read spectrophotometrically (Peirkin Elomer Lamdae 359 UV/Vis/NIR). The maximum absorption was first verified by scanning freshly prepared DPPH from 200 to 800 nm by using the scan mode of the spectrophotometer. Both Butyl hydroxytoluene and ascorbic acid were used as the positive control and standard. The graph of radical scavenging activity (EC_{50}) percentage against extract concentration of 50% was calculated and the inhibition of free radical DPPH in percent (I%) was then calculated by using the following formula:

$$\text{Radical Scavenging Activity}(I\%) = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where :

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

2.3.2 Determination of total reducing power

Total reducing power was determined according to the procedure described by Ferreira et al. (2007) and Woldegiorgis et al. (2014). Briefly, 1 ml of the extract at concentrations ranged from 2 to 12 mg/ml, potassium hexacyanoferrate solutions (1% v/ m, 2.5 ml) and phosphate buffer (0.2 M, pH 6.6, 2.5 ml) were mixed in a test tube. The mixed samples were incubated for 20 min at 50°C. Then, to collect the upper layer of the solution, 2.5 ml trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 min. The upper layer (2.5 ml) was transferred into another tube. Then, it was mixed with 2.5 ml deionized water and 0.5 ml ferric chloride (0.1%) and left to react for 10 min. Finally, at 700 nm the absorbance of the reaction mixture was measured. BHT was used as positive control. The higher reducing power of the antioxidant was shows the stronger absorbance. The graph of absorbance at λ = 700 nm of the extract concentration providing 0.5 of absorbance (EC_{50}) was calculated against extract concentration.

2.3.3 Determination of chelating effects on ferrous ion

Metal chelating effects on ferrous ions was determined according to Woldegiorgis et al. (2014). Briefly, two milliliters of concentrations from 0.05 to 1.5 mg/ml of the extracts in methanol was added to a solution of one mM $FeCl_2$ (0.05 ml). Five mM ferrozine (0.2 ml) was added to initiate the reaction. Methanol was used to adjust total volume to five milliliters and then, the mixture was shaken and left at room temperature for ten minutes. At 562 nm the absorbance of the solution was measured and higher ferrous ion chelating capacity was indicated by a lower absorbance. A 2, 2-bipyridyl, disodium ethylenediaminetetracetate (EDTA) was used as a positive control. Finally, from the graph of ferrous ion 50% inhibition (EC_{50}) against extract concentration was calculated. The inhibition percentage of ferrozine Fe^{2+} complex formation was then calculated by using the following formula:

$$\text{Metal Cheleting Effect (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

Where :

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

2.3.4 Determination of ABTS radical scavenging activity

The ABTS radical scavenging activities were determined using the method of Nishaa et al. (2012). Briefly, the ABTS radical cation was produced by the reaction between 5 ml of 14 mM ABTS solution and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 hrs until the reaction was completed and the absorbance was stable. Prior to use in the assay, the ABTS radical cation was diluted with 50% methanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract (0.2 ml)at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured immediately. As for the antiradical activity, ABTS scavenging ability was expressed as EC_{50} (anti-radical activity), the concentration necessary for 50% reduction of ABTS (Gülçin et al., 2011). The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS (\%)} = \frac{(A_0 - A_1)}{A_0} \times 100\%$$

ABTS radical scavenging activity

Where :

A_0 is the absorbance of the control

A_1 is the absorbance of the sample

2.4 Determination of total phenolics content

Total phenolic content was determined using gallic acid as a standard for the calibration curve based on procedures described by Ferreira et al. (2007), About 1 ml of sample (2000 µg) was mixed with 1 ml of Folin Ciocalteu's phenol reagent. After 3 min, to

adjust to 10 ml with distilled water 1 ml of saturated sodium carbonate (20%) solution was added to the mixture. The reaction was kept in the dark for 90 min and then the absorbance was measured spectrophotometrically at 725 nm using a UV-VIS Spectrophotometer (Agilent Cary Corporation, 1001, Kyoto, Japan). Gallic acid was used to construct the calibration curve ranged from 0.5 to 100 µg/ml (Absorbance= 427.63 gallic acid µg + 0.1453, R^2 = 0.999 and Absorbance= 311.77 gallic acid µg + 0.1107, R^2 = 0.993 which was conducted in different day for pod and seed accession, respectively). The total phenolic content (milligram of gallic acid equivalents per gram) in each extract in Gallic Acid Equivalent (GAE) was calculated by the following formula:

$$\text{Total Phenolic Content (mg/g)} = \frac{\text{c x v}}{\text{m}}$$

Where:
-c is the concentrate ion of gallic acid obtained from the calibration n curve in mg/g
-v is the volume of sample extract in litres
-m is the weight of sample extract in grammes

2.5 Determination of total flavonoids content

Total flavonoid content was carried out by a colorimetric method established by Xu & Chang (2007) and Woldegiorgis et al. (2014). About, 1.25 ml of distilled water and 75 µl of a 5% $NaNO_2$ solution was mixed with 0.25 ml of sample (50 mg). Then, after 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. After 0.5 ml of 1 M NaOH and 2.5 ml of distilled water were added and incubated at room temperature for 5 min. The incubated sample was then thoroughly vortexed for 5 min. At 510 nm the absorbance of the pink color was measured against the blank. For calibration curve (+)-Catechin was used with a concentration range of 10-1000 µg/ml (Absorbance = 27.05 catechin µg + 0.1453, R^2 = 0.995 and Absorbance = 24.57 catechin µg + 0.0404, R^2 = 0.994 which was conducted in different day for pod and seed accession, respectively). The total flavonoid content (milligram of (+)-catechin equivalent (CE) per gram) of each extract was calculated by the following formula:

$$\text{Total Flavonoid Content (mg/g)} = \frac{\text{c x v}}{\text{w}}$$

Where:
-c is the concentration of (+) – catechin obtained from the calibration n curve in mg/g
-v is the volume of sample extract in litres
-m is the weight of sample extract in grams

2.6. Statistical analysis

The experimental result of three parallel measurements were used by Completely Randomised Design (CRD). One way analysis of variance (ANOVA) were used to evaluate data. Duncan's multiple range test ($p<0.05$) by using SPSS version 20.0 for windows were used to separate means and expressed as mean ± standard error (SE). Effective concentration at 50% (EC_{50}) graphs of the respective antioxidant activities were constructed by using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1 Free radical scavenging activity

DPPH *(2,2'-diphenyl-1 - picrylhydrazyl)* is stable radical compound frequently used to examine free radical scavenging activity of natural compounds (Amarowicz et al., 2004). The DPPH radical has a strong absorbance at 517 nm due to its unpaired electron and giving the radical a purple color. But upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH-H (Gursoy et al., 2010). The result of free radical scavenging activity of methanolic extracts of the pod and seed of eight okra accessions and positive controls are shown in Figure 1a and 1b, respectively. The synthetic antioxidant of Butylated hydroxytoluene (BHT) and L-ascorbic acid were used as positive control at the same concentration. The percentage inhibition of free radical scavenging activity of methanolic extracts of the pod and seed accessions were evaluated at concentrations of 2-12 mg/ml.

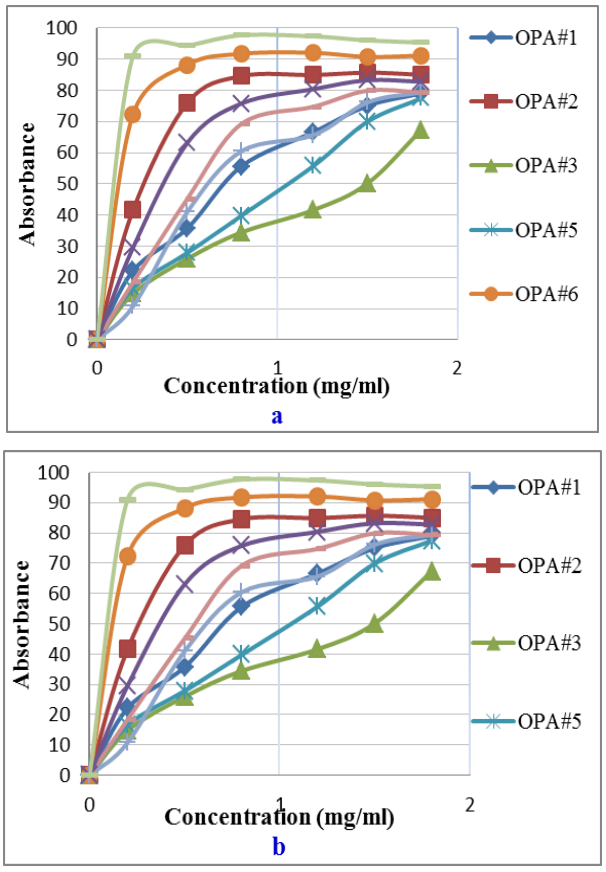


Figure 1 Free radical scavenging of methanolic extract of okra a) pod accessions and b) seed accessions and control

3.2 Reducing power

Reducing power is a novel antioxidation defense mechanism that affects the property of electron transfer ability and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod et al., 2007). The Fe^{3+}–Fe^{2+} reducing power of the methanolic extract may serve as a significant indicator of its potential antioxidant activity (Dastmalchi et al., 2007). The reducing power of the methanolic extracts of the pod and seed accessions were evaluated at concentrations of 1-6 mg/ml. Figure 2a and 2b shows the reducing power of methanolic extracts of okra pods and seeds, respectively. The reducing power of these extracts was observed in a concentration dependent manner. In the present study, the highest reducing power was shown by pod accession OPA#6 with mean absorbance of 0.84 at 4 mg/ml, which is in agreement with its higher DPPH scavenging effect of the pod accession. To the contrary, OPA#8 pod accession was with the least reducing power effect with mean absorbance of 0.48 at 4 mg/ml. The absorbance of reducing power of synthetic antioxidant BHT was 0.97 at 4 mg/ml concentration.

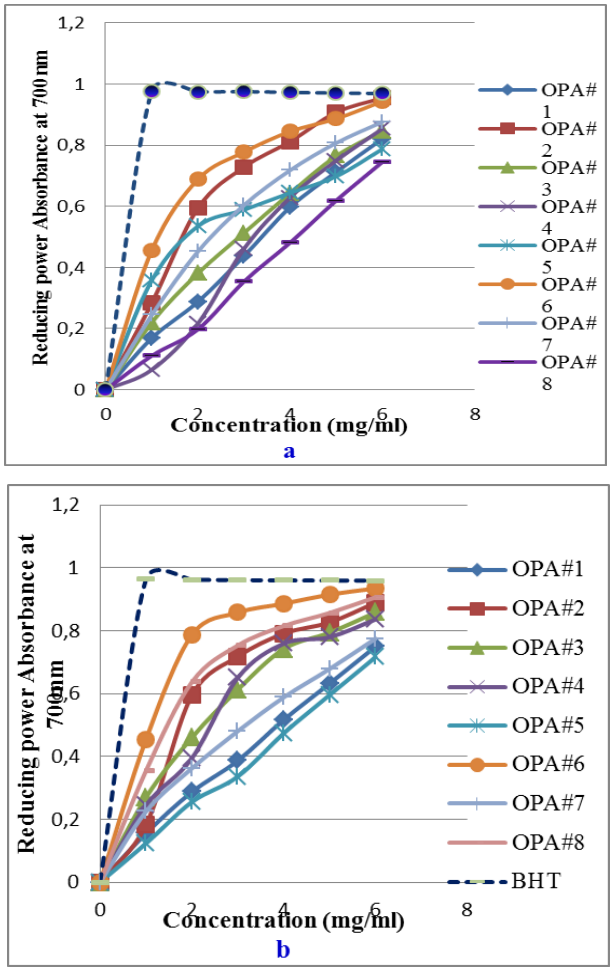


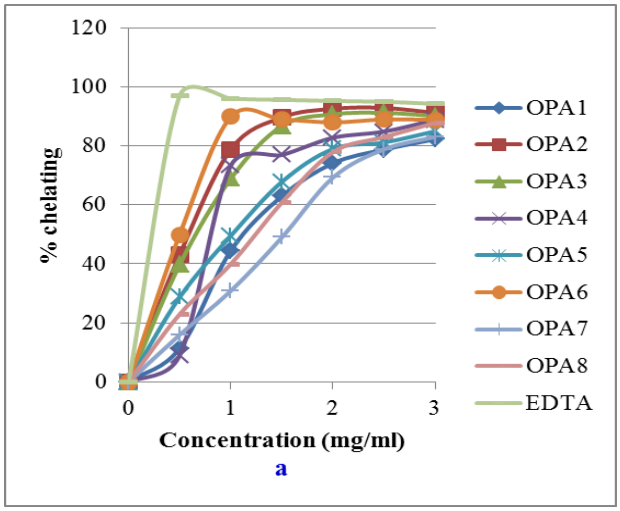
Figure 2 Reducing power of methanolic extracts of eight okra a) pod accessions and b) seed accessions and control

In the seed accessions, the reducing power was higher for OPA#6 with mean absorbance of 0.88 at 4 mg/ml, which is in agreement with its higher in pod accession. Of all the samples analyzed, OPA#5 seed accession was the least reducing power with mean absorbance of 0.47 at 4 mg/ml. The reducing power of the synthetic antioxidants BHT was 0.96 at 4 mg/ml. This result revealed that the reducing power of okra seed and pod accession of OPA#6 is higher than the rest of the accessions and close to the synthetic antioxidants. The higher reducing power activities can be

attributed to higher amounts of polyphenolics and the reducing capacity of a compound may reflect its antioxidant potential (Lee et al., 2007). The reducing power property indicated that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod et al., 2007).

3.3 Chelating effect

Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food (Woldegiorgis et al. 2014). Ferrous ions, the most effective pro-oxidants, are commonly found in food systems. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in the reduction of the intensity of the red colour of the complex (Chandran et al., 2013). Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator (Middha et al., 2013). In the present study, the chelating ability of the okra seed and pod extracts towards ferrous ions was investigated by measuring the interference of the extract with the formation of ferrous and ferrozine complex. The chelating effect of the pod and seed of eight okra accessions and positive control (EDTA) are shown in Figure 3a and 3b, respectively. Quantification of EDTA equivalent metal chelator has given a clear indication that these extracts can effectively chelate metal ions thereby reducing the harm of such metal radicals (Chandran et al. 2013). The optimized concentration range chosen to compare chelating power and to interpolate the EC_{50} (effective concentration at which 50% Fe^{2+}/ferrozine complex is inhibited) is from 0.5 to 3 mg/ml. Similar to DDPH scavenging and reducing power assay, the trend of chelating effect of the methanolic extract increased with concentration and was high for okra pod accession OPA#6 with 89.81% chelating at 1 mg/ml concentration. On the contrary, the pod accession OPA#7 was the least chelating effect with 30.81% at 1 mg/ml concentration. The chelating effect of synthetic antioxidant EDTA was 95.91 at 1 mg/ml concentration.



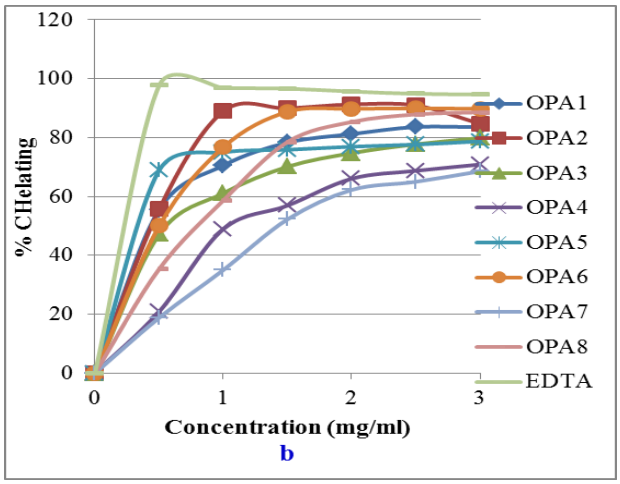


Figure 3 Chelating effect of methanolic extract of eight okra a) pod accessions and b) seed accessions and control

In the seed accessions, the chelating effect of OPA#2 was higher with 88.95% chelating effect whereas seed accession OPA#7 was lower with 35.02% chelating effect at 1 mg/ml concentration.

The radical scavenging activity of all the seed extracts was lower than that of EDTA with 96.87% chelating effect. Ferrous ions could stimulate lipid peroxidation by Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$) in which iron participates as a catalyst in body (Li et al., 2011) and also accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and perpetuate the chain

reaction of lipid peroxidation (Torreggiani et al., 2005). Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions (Lin et al., 2014).

3.4 ABTS radical scavenging activity

ABTS (*2, 2'-azino-bis 3-ethyl-benzothiazoline -6-sulfonic acid)* radical scavenging activity is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxyl radicals) (Leong & Shui 2002). Awika et al. (2003) also have reported the superiority of the ABTS assay because it is operable over a wide range of pH, inexpensive and more rapid. The ABTS radical scavenging activity of the pod and seed of eight okra accessions and reference compounds (L-Ascorbic acid) are shown in Figure 4a and 4b, respectively.

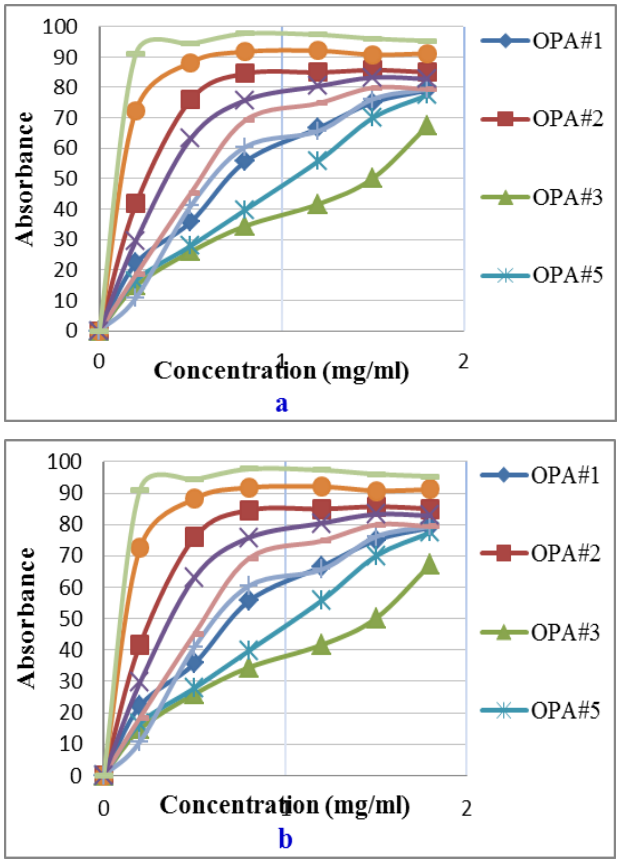


Figure 4 ABTS radical scavenging of methanolic extract of eight okra a) pod accessions and b) seed accessions and control

The percentage inhibition of ABTS radical scavenging activity of methanolic extracts of the pod and seed accessions were evaluated at concentrations of 0.2-1.8 mg/ml.

The result indicates that pod Accession OPA#2 was the highest with 77.91% inhibition of ABTS, whereas the pod accession OPA#5 was the lower with 27.87% inhibition of ABTS at 0.5mg/ml concentration. The ABTS radical scavenging activity of all the pod extracts was lower than that of BHT and ascorbic acids with 89.31 inhibitions at 0.5mg/ml concentration. In seed accessions, OPA#6 was higher with 88.22% inhibition effect of ABTS whereas accession OPA#3 was lower with 25.99% chelating effect at 0.5 mg/ml concentration. The radical scavenging activity of all the seed extracts was lower than that of Ascorbic Acid with 94.31% chelating effect.

3.5 Effective concentrations (EC_{50})

The effective concentrations (EC_{50}) values for DDPH scavenging, reducing power, chelating effects and ABTS scavenging of methanolic extract of each okra pod and seed accessions and positive controls are shown in Table 1.

The EC_{50} values ((mg/ml) of the okra pod accessions were varied and ranged: DDPH scavenging (2.10-10.30); reducing power (1.20-4.20); chelating effect (0.50-1.52) and ABTS scavenging (0.31-1.33). The result of this study revealed that okra pod accession OPA#6 had better antioxidant properties with lower EC_{50} values of 2.10, 1.20, 0.50 and 0.35 mg/ml for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively. In the contrary, okra pod accession OPA#5 had the least antioxidant activity with the highest EC_{50} of 10.30, 1.64, 1.00 and 1.08 mg/ml for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively.

The synthetic antioxidant (BHT, L-Ascorbic acid and EDTA), which was used as a positive control, had a superior performance with the least EC_{50} in all the assays, except for okra pod OPA#6.

Table 1 EC_{50} values (mg/ml) of eight okra pod and seed extracts in the antioxidant evaluation assays

Accessions	DPPH Scavenging (EC_{50}[a])		Reducing Power (EC_{50}[b])		Chelating Effect (EC_{50}[c])		ABTS scavenging (EC_{50}[d])	
	Pods	Seeds	Pods	Seeds	Pods	Seeds	Pods	Seeds
OPA#1	5.30	6.2	3.40	4.00	1.10	0.41	0.70	0.71
OPA#2	4.10	>12	1.63	1.74	0.60	0.42	0.31	0.27
OPA#3	6.50	10.8	2.85	2.20	0.65	0.52	0.64	1.5
OPA#4	3.40	8.0	3.20	2.40	0.80	1.11	0.46	0.38
OPA#5	10.30	>12	1.64	4.30	1.00	0.32	1.08	0.07
OPA#6	2.10	3.9	1.20	1.18	0.50	0.50	0.35	0.13
OPA#7	5.10	>12	2.30	3.20	1.52	1.41	1.33	0.62
OPA#8	8.60	3.1	4.20	1.50	1.35	0.80	0.60	0.55
Average	5.68	8.51	2.55	2.57	0.94	0.69	0.68	0.53
BHT	0.80	1.0	0.43	0.43	-	-	-	-
L-Ascorbic Acid	0.90	0.9	-	-	-	-	0.10	0.10
EDTA	-	-	-	-	0.22	0.22	-	-

[a]EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged. [b]EC_{50} (mg/ml): effective concentration at which the absorbance is 0.5. [c]EC_{50} (mg/ml): effective concentration at which 50% Fe^{2+}/ferrozine complex are inhibited. [d]EC_{50} (mg/ml): effective concentration at which 50% ABTS radicals are scavenged.

The EC_{50} values (mg/ml) of the seed accessions were varied and ranged: DDPH scavenging 3.1->12, reducing power 1.18-4.00, chelating effect 0.32-1.41 and ABTS scavenging 0.07-1.50. Similar to the pod accession, seed accession OPA6 had better antioxidant properties with lower EC_{50} values of 3.9, 1.18, 0.50 and 0.13 mg/ml for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively. Whereas okra seed OPA#7 had the least antioxidant activity with the highest EC_{50} of >12, 3.2, 1.41 and 0.62 mg/ml for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively. Like in that of the pod accession, the synthetic antioxidant (BHT, L-Ascorbic acid and EDTA), which was used as a positive control, had a superior performance with the least EC_{50} in all the assays, except for okra seed accession OPA#6.

The mean of EC_{50} values (mg/ml) of the pod accession is 5.68, 2.55, 0.94 and 0.68 for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively, whereas the mean of EC_{50} values (mg/ml) of the seed accession is 8.51, 2.57, 0.69 and 0.53 for DDPH scavenging, reducing power, chelating effect and ABTS scavenging, respectively. Over all, okra pods had better antioxidant properties for DDPH scavenging and reducing power with low EC_{50} values than the seed accessions. However, chelating effect and ABTS was higher in the seeds with relatively low EC_{50} values (mg/ml) than the pod accessions. Comparatively, the mean EC_{50} values (mg/ml) of the pod and seed accession is far higher that the synthetic antioxidant (i.e. the synthetic antioxidant contain much higher antioxidant activity than the mean of the pods and seeds).

3.6 Total phenolics and Total flavonoids

Natural phenolics exert their beneficial health effects mainly through their antioxidant activity (Fang et al., 2002). These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to nonradical species, and breaking chains to prevent continued hydrogen abstraction from substances (Ozsoy et al., 2008; Vadivel & Biesalski, 2012). In this study, the total phenolic contents of okra pod and seed accessions were expressed in gallic acid equivalent in dry weight bases. Plant extracts containing high levels of gallic acid or any compound equivalent to that may be able to scavenge excessive free radicals such as superoxide anion radicals and peroxy radicals in the human body and protect human cells or

tissues against oxidative stress (Yan et al., 2006; Rangkadilok et al., 2007). Table 2 shows the total phenolic contents of the eight accessions of okra pod and seed extracts. Total phenolic content (mg GAE/g) of the extract was between 28.10 to 95.21 in the pods and 21.28 to 57.34 in the seed accessions. Okra pod accession OPA#6 (95.21 mg GAE/g), OPA#1 (92.38 mg GAE/g) and OPA#8 (92.26 mg GAE/g) were significantly ($P<0.05$) higher in total phenolic content whereas pod accession OPA#7 was the lowest (28.10 mg GAE/g).

Similar to the pod extract, in seed extracts, accession OPA#6 (57.34 mg GAE/g) had significantly ($P<0.05$) higher in total phenolic content and was followed by OPA#7 (52.98 mg GAE/g), OPA#8 (49.03 mg GAE/g), OPA#4 (44.07 mg GAE/g). However, the accession OPA#5 (21.28 mg GAE/g) exhibited the lowest total phenolic content on a dry weight basis. The mean of total phenolic content of the pod accessions (68.21 mg GAE/g) were higher than the seed accessions (40.04 mg GAE/g dw). The mean of total phenol content (40.04 mg GAE/g) of the seed accessions was higher than the value reported for okra seed flour of full fat (25.24 mg GAE/g) by (Adetuyi & Komolafe, 2011). In addition, the mean of total phenolic composition (68.21 mg GAE/g) of okra pod was higher than the common fruits and vegetables reported for their relatively high phenolic constituents (mg GAE/100 g) such as cranberries (52.72±2.15), apple (29.63±0.64), strawberries (16.00±0.12), pineapple (9.43±0.15), banana (9.04±0.32), lemon (8.19±0.35), orange (8.12±0.11), pear (7.06±0.16), and grape (4.96±0.26) (Wojdyło et al., 2007). Sreeramulu & Raghunath (2010) evaluated the antioxidant activity and phenolic content of nineteen vegetables commonly consumed in India and okra fruits were also highest and ranked on the third place according to their phenolic content (167.70 mg GAE/100 g), behind red cabbage and broad beans.

Flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases (Baba & Malik, 2015). Flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species and up-regulate and protect antioxidant defenses (Agati et al., 2012). The total flavonoids content of pod and seed of eight okra accessions are shown in Table 2. The total flavonoid content in the pods varied from 8.18 mgCE/g to 18.72 mg CE/g while in the seeds it varied from 10.73 mg CE/g to 29.04 mg CE/g. The pod of OPA#3 (13.90 mg CE/g) was significantly ($P<0.05$) higher in total flavonoid content and was followed by OPA#6 (17.09 mg CE/g), OPA#1 (16.45 mg CE/g) in that order. However, the accession OPA#7 (8.18 mg CE/g) recorded the lowest total flavonoid content on dry weight basis. In the seeds, the total flavonoid content of accession OPA#6 (29.04 mg CE/g) was significantly ($P<0.05$) higher while accession OPA#3 (10.73 mg CE/g) was the lowest on dry weight basis. Mean total flavonoid contents were 13.66 mg CE/g and 19.59 mg CE/g for accessions of pod and seed, respectively.

Table 2 Total phenolics and flavonoids of the methanolic extract of eight okra pod and seed accessions

Accessions	Total phenols (mg GAE/g)		Total flavonoids (mg CE/g)		Flavonoid/Phenolic	
	Pods	Seeds	Pods	Seeds	Pods	Seeds
OPA#1	92.38±2.29^{a}	27.15±0.01^{e}	16.45±0.07^{c}	21.35±0.04^{d}	0.18	0.79
OPA#2	73.14±0.08^{c}	28.97±1.01^{e}	13.90±0.04^{d}	11.66±0.04^{g}	0.19	0.40
OPA#3	44.12±0.05^{d}	39.48±2.30^{d}	18.72±0.04^{a}	10.73±0.03^{h}	0.42	0.27
OPA#4	79.28±0.02^{b}	44.07±0.02^{c}	11.91±0.49^{e}	24.22±0.22^{c}	0.15	0.55
OPA#5	41.25±0.05^{d}	21.28±0.02^{f}	8.79±0.02^{f}	13.18±0.03^{f}	0.21	0.62
OPA#6	95.21±0.04^{a}	57.34±1.17^{a}	17.09±0.03^{b}	29.04±0.03^{a}	0.18	0.51
OPA#7	28.10±0.05^{e}	52.98±0.13^{b}	8.18±0.08^{g}	26.62±0.04^{b}	0.29	0.50
OPA#8	92.26±1.69^{a}	49.03±2.35^{b}	14.29±0.04^{d}	19.91±0.03^{e}	0.15	0.41
Average	68.21	40.04	13.66	19.59	0.20	0.49

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

This finding revealed that the mean amount of total phenolic in pods accession is higher than the total flavonoid content of the pod accessions. However, this finding disagree with the result obtained by Ahiakpa et al. (2013), in which okra pod possesses high amounts of total flavonoids as well as moderate amounts of total phenolics.

4. CONCLUSIONS

In this study, eight selected okra pod and seed accessions were evaluated for their phenolic contents and antioxidant activities. The results showed that phenolic contents and antioxidant activity levels were varied widely across pod and seed accessions. It has been also shown that the antioxidant levels of the pod and seed accessions increased with the increasing concentration of the samples and were dependent on the extract concentration. In general, the Ethiopian indigenous okra pod and seed accessions evaluated for the first time indicated it as a new source of natural antioxidants. Particularly, both okra pod and seed accession, OPA#6 is a potentially rich source of antioxidants that could be used in food industry and replace synthetic antioxidants.

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