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Antioxidative Activities and Phenolic Content of Extracts from Okra (*Abelmoschus esculentus* L.)

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Abstract: The aim of this study was to determine the antioxidant activities, TPC and to identify phenolic compounds of seeds and pulped okra (*Abelmoschus esculentus* L.), a vegetable that is consumed worldwide because it plays an important role in the human diet by supplying carbohydrates, minerals and vitamins. The antioxidant activities of seeds and pulped okra were evaluated by DPPH radical scavenging and ABTS radical cation decolorization assays were used. Total Phenolic Content (TPC) was determined using the folin-ciocalteu assay. I dentification of phenolic compounds was achieved using HPLC with the UV-diode array detection. The TPC of pulped and seeds of okra extracts were 10.75±0.02 mg GAE/100 g e xtract and 142.48±0.02 mg GAE/100 g extract which corresponding with scavenging activities. The predominant phenolic compound was procycanidin B2, following procycanidin B1 and rutin in seeds but in pulped okra they were identified as catechin, procycanidin B2, epicatechin and rutin. These findings in associations with the high content of antioxidant activity and phenolics in okra enhance the importance of this food stuff for the human diet.

Key words: Antioxidant activity, folin-ciocalteu assay, phenolic content, okra, *Abelmoschus esculentus* L., Thailand

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is an important vegetable which is widely distributed from Africa to Asia, Southern European and America. Okra plays an important role in the human diet by supplying carbohydrates, minerals and vitamins: K, Na, Mg and Ca were found to be the principal elements, with Fe, Zn, Mn and Ni also present (Moyin-Jesu, 2007; Arapitsas, 2008). Okra seeds could serve as alternate rich sources of protein, fat, fiber and sugar (Oyelade *et al*., 2003; Deters *et al*., 2005; Adelakun *et al*., 2009a). The natural phenolic content of okra seeds has been reported (Huang *et al*., 2007; Arapitsas, 2008; Adelakun *et al*., 2009b).

Recently they have received much attention since many epidemiological studies suggest that consumption of foods rich in phenolic compounds is associated with reduced risk of cardiovascular diseases, stroke and certain forms of cancer (Hollman and Katan, 1999; Jerez *et al*., 2007; Klimczak *et al*., 2007; Kubola and Siriamornpun, 2008; Du *et al*., 2009). Phenolic compounds have recently gained significant interest among various antioxidants. Structure activity relationship studies of flavanoids have showed that the dissociation of hydroxyl function occurs in the following sequence: 7-OH> 4-OH> 5-OH. The o-hydroxyl structure in the B ring, the 2,3-double bond in conjugation in the C ring and the 3- and 5-OH groups with the 4-oxo function in A and C rings are essential for effective free radical scavenging activity (Lien *et al*., 1999; Erlund, 2004). The objective of this study was to determine the antioxidant activities, Total Phenolic Content (TPC) and to identify phenolic compounds of seeds and pulped okra. The antioxidant properties of okra extracts were tested for their total antioxidant capacity with two (DPPH and ABTS) different method, TPC by folin-ciocalteu method and identification of phenolic compounds using the RP-HPLC method.

MATERIALS AND METHODS

Chemicals: Acetonitrile, ethanol, hexane methanol and phosphoric acid purchased from Merck (Darmstadt,

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Germany). The chemicals used for determination of antioxidant activities and total phenolic contents included 2, 2-diphenyl-2-picrylhydrazyl Hydrate (DPPH), gallic acid, folin-ciocalteu's reagent, ABTS, potassium persulfate and sodium carbonate (Na_2CO_3) which were obtained from Fluka chemical and trolox was obtained from Sigma-Aldrich. Chemicals employed for determination of phenolic compound standards included rutin, procyanidin B1 and procyanidin B2 which purchased from Sigma-Aldrich and catechin, epicatechin, gallic acid and trolox, quercetin were obtained from Fluka chemical.

Plant materials: Okra (*Abelmoschus esculentus* L.) samples were collected from a local market in Maha Sarakham province located in Northeastern Thailand . The okra was thoroughly cleaned with distilled water several times. After that seed and pulp were separated prior freeze-drying and ground into a fine powder and stored in sealed plastic bags at -20°C until extraction.

Powder samples were extracted by soaking in hexane for 60 h in a soxhlet extraction at room temperature followed by filtration with Whatman No. 1 filter paper. The residues were re-extracted with methanol solvent for a total of 2 extractions. The extracts were evaporated under a vacuum at 50°C using a rotary evaporator. After extraction with hexane, the residue was transferred for extraction with methanol in the same manner as the hexane extract.

The crude extracts were stored in the dark in a vial and kept at 4°C until further tests to determine the antioxidant activity, total phenolic contents and HPLC analysis. Each evaporated thick and viscous extract (0.020 g±0.001 mg) was diluted with 25 mL methanol.

Extraction yield: The extraction yield was analyzed by the method of Liu and Yao (2007) with some modifications. Briefly, the filtrate was evaporated to dryness using a rotary evaporator under a vacuum at 60°C. The yield of dried power, based on a percentage of dry weight was then calculated from equation as following Eq. 1:

$$\text{Yield}\ (\%) = (W_1 \times 100)/W_2 \qquad (1)$$

where, W_1 was the weight of extract after evaporation of methanol and W_2 was the dry weight of sample. These extracts were used for determination of both antioxidant capacity and total phenolic content.

Determination of antioxidant activity: The antioxidant activities of seeds and pulped okra fruit was evaluated by DPPH free radical scavenging assay and ABTS radical cation decolorization assay.

DPPH radical scavenging assay: The free radical scavenging activity of the extract was measured using the DPPH assay following the method from Liu *et al.* (2008).

Briefly, 1 mL of 0.2 mM DPPH radical solution in ethanol was mixed with 1 mL of the extract sample solution at different concentrations. Then, the samples were left to stand in a dark room at room temperature for 20 min. Absolute ethanol 1 mL was mixed with 1 mL of DPPH to be used as a blank. The absorbance was measured at 515 nm using a UV-visible spectrophotometer for triplicate measurements.

All samples were analyzed in triplicate. Trolox equivalent was used as standard reference. The percentage of remaining DPPH against the sample concentration was plotted to obtain the amount of antioxidant (μg) necessary to decrease free radicals by 50%. A smaller IC_{50} value corresponds to a higher antioxidant activity.

ABTS radical cation decolorization assay: The assay as detailed by Francisco and Resurreccion (2009) was based on the relative ability of antioxidants to scavenge the radical cation of 2,2′-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$). It was generated by reacting ABTS (7 mM) with potassium persulphate (2.45 mM). The mixture was allowed to stand in the dark at room temperature for 16 h before use. The ABTS was dissolved and diluted in ethanol. About 1 mL of trolox standard solution or extract was mixed with 100 μL of $ABTS^{\bullet+}$. The absorbance was measured at 734 nm. The results were expressed as Trolox Equivalent Antioxidant Activity (TEAC).

Total phenolic content: Total Phenolic Contents (TPC) of seed and pulp from okra fruit extracts were determined using the folin-coicalteau assay, as modified by Jerez *et al.* (2007). The extract solutions (0.5 mL) were mixed with 2.5 mL of 10% folin-ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate. The mixtures were agitated with a vortex mixer and allowed to stand at room temperature for 30 min in the dark. The absorbance of extracts and prepared blank were measured at 765 nm using UV-visible spectrophotometer (Perkin-Elmer Model Lambda 25, USA). Quantification of Total phenolic was based on a gallic acid standard curve generated by preparing 0-100 μg L^{-1} of gallic acid. The TPC were expressed as milligrams of gallic acid equivalents (GAE)/100 g extract.

RP-HPLC separation of extracts: The HPLC analysis was performed using the Shimadzu HPLC system equipped with LC-10 Advp liquid chromatography, SPD-m10Avp

diode array detector, SIL-10Advp auto-injection, SCL-10Advp system controller and CTO-10Avp column oven. Chromatographic separation was performed on a C_{18} column (4.6 mm×25 cm, 5 μm). The solvent system used was a gradient of the mobile phase, a mixture of solution A which contained 2% acetonitrile and 0.2% phosporic acid and solution B which contained a mixture of 97.8% acetonitrile and 0.2% phosporic acid at a constant flow rate of 0.6 mL min^{-1}.

The following gradient was used: 0-30 min from 50% A, 20 B-80 A, 50% B with a flow rate of 0.6 mL min^{-1}; 30-35 min from 40 A, 50 B-50 A, 60% B with a flow rate of 0.6 mL min^{-1}; 35-40 min from 40 A, 50 B-50 A, 80% B with a flow rate of 0.6 mL min^{-1}; 40-55 min from 80 A, 20 B. Operating conditions were as follows: column temperature, 40°C; injection volume, 20 μL; UV-diode array detection at 254 nm.

RESULTS

Extraction yield: The yields from seeds and pulp of *Abelmoschus esculentus* L. from methanol extraction are shown in Table 1. The extraction yields of the samples were 1.97 and 3.53% for seeds and pulp, respectively. The yield from the hexane extract was very low.

Total Phenolic Contents (TPC): Total phenolic content (TPC), expressed as gallic acid equivalent/100 g extract. The pulp of *Abelmoschus esculentus* L. presented the lowest amount of TPC (10.75 ± 0.02 mg GAE/100 g extract) while a higher value was observed from the seeds 142.48±0.02 mg GAE/100 g extract (Table 1).

Antioxidant activities: The antioxidant activity of *Abelmoschus esculentus* L. was evaluation by DPPH and ABTS methods and the results are shown in (Table 1). Both methods showed that pulp and seeds of *Abelmoschus esculentus* L. had high relative antioxidant activity. The concentration that provided 50% radical scavenging (IC_{50}) was determined (Dutra *et al*., 2008).

These differences could be explained by the different mechanisms of the analytical methods. ABTS and DPPH methods are based on the reduction of ABTS and DPPH free radicals in samples but the value from the DPPH assay might be lower than that from the ABTS assay. The results showed that seed of *Abelmoschus esculentus* L. had the higher antioxidant capacity than its pulp and lower trolox from both assay.

The relationship between the antioxidant capacity and TPC analysis was highly significant as shown in Table 1. These indicate that the phenolic compounds could be the main cause of antioxidant power of plant samples, in accordance with the previous finding that many phenolic compounds in plants are good sources of natural antioxidants.

Table 1: Results of yield and antioxidant activity from DPPH and ABTS assays of extracts from *Abelmoschus esculentus* L.

Sample part	Yield (%)	TPC (mg/100 g extract)	IC_{50} (mg mL^{-1}) DPPH assay	IC_{50} (mg mL^{-1}) ABTS assay
Seed	1.97±0.27	142.48±0.02	44.1	7433
Pulp	3.53±0.43	10.75±0.02	5573.0	24910
Trolox	-	-	17.6	236

Table 2: Content of phenolic compounds from extracts of *Abelmoschus esculentus* L.

Phenolic compounds	Phenolic compounds of *Abelmoschus esculentus* L.(mg/100 g extract) Seeds	Pulp
Catechin	ND 56.00±0.01	-
Epicatechin	ND 31.90±0.01	-
Procyanidin B1	289.40±0.03	ND
Procyanidin B2	675.20±0.01	26.50±0.01
Quercetin	25.90±0.01	ND
Rutin	0.32225.60±0.01	1.50±0.01

ND = Not Detect

RP-HPLC for analysis of phenolic compounds: In the present study, the qualitative and quantitative analyses of the methanol and acetone extracts from seeds and pulp of *Abelmoschus esculentus* L. using RP-HPLC are shows in Table 2, with the chromatograms with detector responses at 254 nm. The results showed that phenolic compounds found in the extracts included catechin, epicatechin, procyanidin B1, procyanidin B2, quercetin and rutin which identified by comparisons to the retention times and UV spectra of standards analyzed under identical analytical conditions while the quantitative data was calculated from their respective calibration curves.

The RP-HPLC assay for phenolic compounds was found to be linear in the range of 0.5-100 ug mL^{-1} with high (0.9941-0.9999) correlation coefficients (R^2).

Procyanidin B2 was the predominant phenolic compound in seeds of *Abelmoschus esculentus* L., the mean value was 675.20±0.01 mg/100 g extract. The main of phenolic compounds in the pulp were epicatechin, catechin, rutin and procyanidin B2. Catechin and epicatechin were not detected in the seeds while quercetin and procyanidin B2 were not detected in the pulp of *Abelmoschus esculentus* L.

DISCUSSION

A relationship between TPC and IC_{50} value was found the high content of TPC related to good antioxidant capacities. It can be seen that the extracts studied in this research had potential to contain antioxidant substances.

Phenolic compounds are widely distributed in plants which have gained much attention, due to their antioxidant activities and free radical scavenging abilities which potentially have beneficial implications for human health. However, high TPC values did not correspond in a linear fashion to a high antioxidant activity.

Due to folin-ciocalteu reagent not being specific to just phenolic contents but to any other substances that could also be oxidized by the reagent.

CONCLUSION

The flavonoids extracted from seeds and pulped okra were identified as procyanidin B2, procyanidin B1, rutin, quercetin, catechin and epicatechin. The data presented in this study demonstrates that the amounts of phenolic compounds differ significantly between defferent parts of *Abelmoschus esculentus* L.

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