Properties and characterization of antioxidant and antiglycative activities for the multiple harvests of aquatic- and field-cultivated peanut leaves and stems

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ABSTRACT

Peanut top parts of leaves and stems are feasible to be consumed as a vegetable. Peanut kernels of Tainan 9 were aquatic- and field-cultivated for 30 days and the top parts were harvested for three times at a 10-day interval. The highest total phenolics, epicatechin and caffeic acid contents in leaves were detected in the second harvest of aquatic-cultivation. In comparison, higher epicatechin and caffeic acid contents were observed in leaves of aquatic-cultivation than the field-cultivated leaves. The reducing powers, total equivalent antioxidant capacity and inhibitory activities against AGEs formation varied with a close dependence on total phenolics contents. Based on SDS–PAGE analysis, all harvested aquatic- and field-cultivated peanut leaves were effective in inhibition of albumin glycation. It is of merit to demonstrate that multiple harvests of the green top parts of peanuts bear potent antioxidant and antiglycative activities.

1. Introduction

Peanut (Arachis hypogaea L.) is cultivated mainly in the tropical, sub-tropical and warm areas of the earth and consumed world-widely. About 13.5 million ha of peanuts were grown in Asia, 5.3 million ha in Africa, 1.2 million ha in Americas and 0.1 million ha in other parts of the world (Stalker, 1997). Peanuts are mostly grown for harvest of the pods and/or shelling to collect the kernels for food use. Although it is not conventional, to harvest leaves and stems of the juvenile peanut plants as green vegetable for humans or feeds for livestock is of worth further investigations. In particular, multiple harvests of the top parts to increase per plant biomass production deserve research interest.

In addition to contribution of macro-nutrients, various bioactive phytochemicals including phenolic acids, stilbenes, phytosterols, alkaloids, flavonoids and their derivatives have been isolated from peanut shells, kernels, stems, leaves and roots (Chen, Wu, & Chiou, 2002; Chung, Park, Chun, & Yun, 2003; Lopes, Agostini-Costa, Gimenes, & Silveira, 2011). As determined, phenolic acids especially caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and m-coumaric acid are the prevalent hydroxycinnamic acids in the leafy vegetables (Khanam, Oba, Yanase, & Murakami, 2012). Caffeic acid and...
its derivatives have been detected in mulberry leaves of Tunisian Morus species (Thabti, Elfaleh, Hannachi, Ferchichi, & Campos, 2012). Caffeic acid is a major compound in the leaves of sugarcane (Saccharum officinarum L.) (28NG256 variety) and displays the highest induction of apoptosis in HepG2 cells and the highest increase of ROS generation (Lee, Chen, Yu, Wang, & Duh, 2012). Corchorus olitorius leaf extracts contained caffeic acid and exhibited inhibitory activities on the key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting) (Oboh et al., 2012). The phenolic compounds, including caffeic, ferulic, vanillic, p-hydroxybenzoic and protocatechuc acids have been detected in beet root (Beta vulgaris) pomace. The beet root extracts exhibit potent cytotoxic activities against human cell lines of MCF7 (breast adenocarcinoma) and MRC-5 (fetal lungs) (Vulić et al., 2012). Moreover, caffeic acid improves glucose metabolism by promoting glycogenesis and inhibiting gluconeogenesis in TNF-α-treated insulin-resistant mouse hepatocytes (Huang & Shen, 2012). Epicatechin is a major compound detected in the litchi-flower-water extracts (LFWEs). LFWEs decrease serum lipids and liver lipid accumulation in high-fat-diet fed hamsters and increase hepatic antioxidant capacities as well as decrease liver damage/inflammatory indices, CRP levels and MMP-9 (Chang et al., 2013).

Most of the phytochemicals are belonging to the secondary metabolites biosynthesized by phenylpropanoid pathway as physiological responses to biotic and abiotic stress-elicitations (Dixon, Xie, & Sharma, 2005; Vogt, Pollak, Tarlyn, & Taylor, 1994). Wound-induced biosynthesis of chlorogenic acid, alkyl ferulate esters, cell wall-bound phenolic esters as defense compounds or precursors for synthesis of the wound-induced polyphenolic barriers have been demonstrated (Bernards & Lewis, 1992; Hahlbrock & Scheel, 1989). Cut-to-harvest of the peanut top parts as an abiotic stress of artificial wounding to induce biosynthesis of the phenylpropanoid compounds and increase bioactivities and related health benefits in the following harvests is likely.

A circulating hydroponic system using a nutrient film technique to facilitate aeration has been developed for aquatic cultivation of peanut (Graves, 1983; Hill et al., 1992; Wu et al., 1997). Aquatic floating cultivation system (AFCS) is a method of growing plants on a board floating on aquatic cultivation solutions without artificial aeration or circulation of the aquatic cultivation solutions (Liu, Wen, Chiou, Wang, & Chiou, 2003). In comparison to field-cultivation, AFCS merits mobility of the growing system, flexibility of cultivation solution-selection and allowance of routine checks of the roots. Thus, in this study, peanuts were concurrently aquatic-cultivated in a greenhouse and field-cultivated in an experimental field. The top parts were harvested at a 10-day interval for three times. The yields of harvested leaves and stems and their total phenolics contents as affected by cultivation and different time (order) of harvest were determined. The 80% methanol extracts of the leaves and stems were further subjected to compositional analysis and characterization of antioxidant and anti-glycative activities. SDS–PAGE analysis of the glycated albumins as affected by the leaf extracts was extended.

2. Materials and methods

2.1. Chemicals

Methanol, ethanol, acetic acid, acetone, trichloroacetic acid and potassium persulfate were purchased from J.T. Baker (Phillipsburg, NJ, USA); potassium ferricyanide and ferric chloride were purchased from Showa Chemicals Co., Ltd. (Tokyo, Japan); Ascorbic acid was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan); 2-mercaptoethanol, Folin–Ciocalteu phenol reagent and sodium dodecyl sulfate (SDS) were purchased from Merck (Darmstadt, Germany); Acryl/bis solution, ammonium persulfate (APS), and TEMED were purchased from Amresco Inc. (Solon, OH, USA). Authentic epicatechin and caffeic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Aquatic-cultivation of peanuts and harvest of the top parts

As a primary experiment, three cultivars of peanut (A. hypogae L., Spanish cultivar), namely, Tainan 9, Tainan 11 and Tainan 14, were selected and subjected to aquatic floating cultivation (Liu et al., 2003) with reversed osmotic (RO) water, Murashige and Skoog (MS) solution (Murashige & Skoog, 1962) and 0.1% Hyponex® solution (No. 1 Hyponex powder, Hyponex Corp., Marysville, OH, USA) as media. Sound seeds were selected and soaked with RO water for 4 h. The kernels were drained and transferred onto a round polyethylene plastic (PE) basket (29 cm diameter and 10.5 cm height) and covered with the wet tissue paper. The PE plastic basket was kept in the headspace room of a round polylon box (29.5 cm diameter and 14.5 cm height). The box base was filled with 600 mL of RO water and covered with round polylon box-lid to keep high humidity. During incubation, the kernels were rinsed with RO water and the tissue paper was changed daily. After 5 days of incubation, each germinated kernel was transplanted into a punctured hole of a round polylon plate (2 cm thickness) floating on 550 mL of RO water, MS solution and 0.1% (w/v) Hyponex® solution in each cup (9 cm diameter and 18.7 cm height) without artificial aeration. All aquatic media were not renewed. Water losses were replenished with RO water to maintain the solution level.

After 30 days of cultivation, the top parts of leaves and stems were harvested, weighed and subjected to forced-air drying at 50°C until constant weight was reached and weighed to determine dry mass yields. The dry material was ground into powder (RT-02, 150G High Speed Grinder, Rong Tsong Precision Technology Co., Taichung, Taiwan) and subjected to solvent extractions with water and 80% methanol and followed by antioxidant and anti-glycative activities determinations. As generally compared, based on biomass production of the top parts, Hyponex® is better than RO water and MS solution for aquatic cultivation and 80% methanol was better than water in extraction of the antioxidant and anti-glycative components. In comparison of the test peanut cultivars, Tainan 9 exhibited the higher antioxidant and anti-glycative activities than those of Tainan 11 and Tainan 14. Accordingly, Tainan 9 was selected and...
aquatic-cultivated with 0.1% Hyponex® solution for multiple harvests and followed characterization.

2.3. Multiple harvests of the aquatic- and field-cultivated peanut leaves and stems

In this study, aquatic- and field-cultivation were concurrently conducted in the early summer. The experimental field is ca. 30 m of distance from the glass greenhouse of Department of Agronomy, National Chiayi University. In the experiment period of 30 days, the daily lowest and highest ambient temperatures were recorded by a dual-temperature thermometer (No. 7265, Sato Keiryoki Mfg. Co., Chiyodaku, Japan) and, as recorded, varied from 20.0 to 44.0 °C. The lowest and highest temperature range during field cultivation varied from 19.0 to 39.0 °C. The average highest temperatures of the aquatic and field cultivation were 37.5 and 32.7 °C, respectively.

As described above, in each of 24 round plastic cups (n = 24) filled with 550 mL of 0.1% (w/v) Hyponex® solution, one 5 day-germinated kernel was transplanted for aquatic-cultivation in the glass greenhouse. After cultivation for 10 days, the top parts of 24 peanut plants were cut (Fig. 1A) to harvest for the first time. Then, the polylon plates with basal stem and root were cultivated further for an additional 10 days and the top parts were cut to harvest for the second time. Similarly, the plates were further cultivated for an additional 10 days to harvest the third time.

From each harvest, the top parts of 24 peanut plants were randomly distributed into three sublots. The leaves and stems from each sublot were further separated (Fig. 1B), pooled, weighed and subjected to drying at 50 °C in a forced-air oven for 48 h and weighed to determine moisture contents and yields of biomass. Each dry sample was ground into fine powder and stored in a PE bag for storage at −20 °C for further characterization.

For field cultivation, sound peanut seeds (n = 30) of Tainan 9 were soaked in water for 4 h and seeded in an experimental field of Department of Agronomy, National Chiayi University with normal cultivation practice (Chiou, Liu, Liu, Ferng, & Tsai, 1992). After 5 days of cultivation to confirm normal germination, 24 peanut plants were selected, divided and labeled into three sublots (eight plants for a sublot). After cultivation for an additional 10 days (counted from the 6th day of seeding), the top parts of each plant in each sublot were cut to harvest (Fig. 1A) and separate leaves and stems (Fig. 1B). The second and third harvests were operated at a 10-day interval and the followed sample preparations were identical to those described above for aquatic cultivation.

2.4. Methanol extraction and analyses of the harvested leaves and stems

From each of the prepared powders, 0.05 g was weighed and deposited in a 10 mL centrifuge tube and mixed with 2.5 mL of 80% (v/v) aqueous methanol solution. The suspension was homogenized by a polytron (PT3000, Kinematica AG, Littau, Switzerland) at 15,000 rpm for 1 min. Then, the homogenate was centrifuged at 15,000 g at 4 °C for 20 min (Sigma Labrozentrifugen 2K15, Osterode, Germany) and the supernatants were withdrawn and stored at −20 °C for further use.

For determination of the total phenolics contents, it was conducted according to the method described by Siddhuraju and Becker (2003). Briefly, the aliquot of extract was deposited in a glass test tube and mixed with Folin–Ciocalteu phenol reagent. After 30 min of incubation, the absorbance at 750 nm against the reagent blank was determined (U-2001 Spectrophotometer, Hitachi Co., Tokyo, Japan). Gallic acid solutions were prepared and used for construction of a standard curve. The results were expressed as mg gallic acid equivalent (GAE)/g of dry mass.

Fig. 1 – Photographs of the harvested top parts and the cutting positions to separate leaves and stems of the harvested top parts of the aquatic- and field-cultivated peanuts harvested at 10-day interval for three times; (A): photographs of the harvested top parts; (B): the cutting positions to separate leaves and stems; A-1: first aquatic-harvest; A-2: second aquatic-harvest; A-3: third aquatic-harvest; F-1: first field-harvest; F-2: second field-harvest; F-3: third field-harvest.
Fig. 2 – Total phenolics content (TPC), antioxidant and antiglycative activities of the aquatic- and field-cultivated peanut leaves and stems harvested at 10-day interval for three times; (A): TPC; (B): reducing power; (C): total equivalent of antioxidant capacity (TEAC); (D): antiglycative activity; AFCS: aquatic cultivation; FIELD: field cultivation; 1: first harvest; 2: second harvest; 3: third harvest; AG: aminoguanidine. TPC: mg gallic acid equivalent (GAE)/g dry mass; reducing power: mg ascorbic acid equivalent (AAE)/g dry mass; TEAC: μmol Trolox equivalent/g dry mass; antiglycative activity was expressed as % inhibition of AGEs formation. The values represent mean ± SD (n = 3). The vertical bars marked with different letter are significantly different (p < 0.05).
For HPLC analysis and identification of the separated compounds, the membrane-filtered (0.45 μm) methanol extracts were subjected to analysis with HPLC equipped with a dual pump (L-7100), a UV–vis detector (L-2420) and a photodiode array detector (PDA, L-7455) (Hitachi Co., Ltd., Tokyo, Japan). A reverse phase C18 column (Hypersil-100, 250 × 4.6 mm, 5 μm) (Thermo Fisher Scientific Inc., Waltham, MA, USA) was run with a gradient mobile phase consisting two solvents: A (methanol with 0.5% acetic acid) and B (water with 0.5% acetic acid). The gradient solvent program was set by varying the proportion of solvent B as 0 min, 100% B; 14 min, 60% B; 20 min, 0% B; 23 min, 0% B; 26 min, 100% B; and 30 min, 100% B. The injection volume, flow rate and monitoring wavelength were 20 μL, 1.0 mL/min and 280 nm, respectively. Two authentic compounds of epicatechin and caffeic acid (Sigma Chem. Co., St. Louis, MO, USA) were run concurrently as reference standards and also used for concentration estimation.

For identification of the separated peaks, the retention times of two peaks (indicated as peak 1 and peak 2 in Fig. 3) were monitored at 280 nm of the extracts of aquatic- and field-cultivated peanut leaves harvested at 10-day interval for three times; AFCS-1: first aquatic harvest; AFCS-2: second aquatic-harvest; AFCS-3: third aquatic-harvest; FIELD-1: first field-harvest; FIELD-2: second field-harvest; FIELD-3: third field-harvest; Peak 1: epicatechin; Peak 2: caffeic acid.

Fig. 3 – HPLC chromatograms monitored at 280 nm of the extracts of aquatic- and field-cultivated peanut leaves harvested at 10-day interval for three times; AFCS-1: first aquatic harvest; AFCS-2: second aquatic-harvest; AFCS-3: third aquatic-harvest; FIELD-1: first field-harvest; FIELD-2: second field-harvest; FIELD-3: third field-harvest; Peak 1: epicatechin; Peak 2: caffeic acid.
were identical to those of epicatechin and caffeic acid, correspondingly. Purity of the substance under either peak 1 or peak 2 was novel as analyzed and evaluated based on their PDA monitoring diagrams. After achieving their UV spectra in the range of 200–400 nm, their spectra were also matched with those of the authentic compounds (Fig. 4). It is assumptive to identify the peaks 1 and 2 as epicatechin and caffeic acid.

2.5. Antioxidant characterization

The reducing power of each sample was determined according to the procedure reported by Yen and Chen (1995) with modification. Briefly, aliquots of 0.5 mL extracts after appropriate dilution was mixed thoroughly with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide solution. All mixtures were incubated in a water bath at 50 °C for 20 min and then rapidly cooled in an ice bath. Into each tube, 0.5 mL of 10% (w/v) trichloroacetic acid solution was added and thoroughly mixed by vortexing. After centrifugation of the tubes (1000 g at 20 °C), 1.0 mL of the supernatant was withdrawn and mixed with 1.0 mL water and 0.2 mL of 0.1% (w/v) ferric chloride solution. Then, the mixed solution was incubated at the ambient temperature (25–28 °C) without light exposure for 10 min and followed by absorbance determination at 700 nm. Ascorbic acid solutions were prepared and determined concurrently to construct a reference curve and used for equivalency estimation.

Trolox equivalent antioxidant capacity (TEAC) was determined mainly based on the scavenging capacity of ABTS free radical cation (ABTS•+) (Madhujith, Izydorczyk, & Shahidi, 2006). According to the modified method of Li, Cheng, Wong, Fan, and Chen (2007), the preformed ABTS•+ was generated by oxidation of 14 mM ABTS aqeous solution with 4.9 mM potassium persulfate for 12–16 h in the dark at ambient temperature (25–28 °C). Prior to determination of TEAC, the ABTS•+ solution was diluted with ethanol to give an absorbance values in the range of 0.70 ± 0.05 at 734 nm. All of extract samples were diluted appropriately to give absorbance values in the range of 20–80% to that of the blank. Aliquot (1.9 mL) of the diluted ABTS•+ solution was added and mixed with 50 μL of each peanut extract or a series of Trolox standards and incubated at ambient temperature for 6 min and followed by absorbance determination at 734 nm. A series of the Trolox solutions were prepared for construction of a reference curve for equivalency estimation.

2.6. Antiglycative activity determination

Determination of the inhibitory activities against AGEs (advanced glycated end products) formation of the peanut extracts was conducted following the reported method of Wang, Chang, Pokkaew, Lee, and Chiou (2011). Briefly, bovine serum albumin solution (BSA, 60 mg/mL containing 0.06% sodium azide) and fructose (1.5 M) were respectively prepared in 0.2 M potassium phosphate buffer (pH 7.4). For each reaction of glycation (BSA solution, fructose solution and peanut plants extract) were reacted at a ratio of 1:1:1 (v/v/v). Aminoguanidine (AG) 3 mM was used as a positive control. A series of the tubes containing above mixed solution were respectively incubated for 24 h at 50 °C in an incubator (MIR 252, Sanyo, Osaka, Japan). After incubation, 100 μL of each reacted solution was withdrawn to a 96-well plate and subjected to intensity determination by a spectrofluorometer (FLx 800, Bio Tek, Winooski, VT, USA). The machine was set at 360 nm of excitation wavelength and 460 nm of emission wavelength. Results were expressed as percentage inhibition of formation of the glycated protein.

Antiglycative activities were further assessed by SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) analysis. The glycated proteins formed after reaction of BSA and fructose at 50 °C for 24 h were subjected to sample preparation and electrophoresis following the previously reported procedure (Wang et al., 2011).
When peanut kernels of Tainan 9 were respectively aquatic-cultivated and field-cultivated for 30 days and the top parts of peanut plants were harvested at a 10-day interval for three times, photographs of the harvested top parts are shown in Fig. 1A. Apparently, sizes of the harvested top parts decreased in order with time of harvest. Their dry mass weights of the separated leaves and stems also decreased in order with time of harvest. This might be relevant to depletion of soil nutrients or requirement of a longer cultivation period. Moisture contents of the harvested leaves and stems varied with cultivation practice and time of harvest (Table 1). In comparison, dry mass weights of the separated leaves and stems also decreased in order with time of harvest. Their dry mass weights of the aquatic-cultivated leaves and stems were higher than those of other harvested parts.

### Table 1 – Yields of dry mass and moisture contents of the aquatic- and field-cultivated peanut leaves and stems harvested at a 10 day-interval for three times.

<table>
<thead>
<tr>
<th>Cultivation and harvest</th>
<th>Yield of dry mass, g/plant$\dagger$</th>
<th>Moisture content, %$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stems</td>
</tr>
<tr>
<td>Aquatic floating cultivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First harvest</td>
<td>0.30 ± 0.01$^b$</td>
<td>0.09 ± 0$^c$</td>
</tr>
<tr>
<td>Second harvest</td>
<td>0.24 ± 0.06$^c$</td>
<td>0.11 ± 0.03$^b$</td>
</tr>
<tr>
<td>Third harvest</td>
<td>0.02 ± 0$^a$</td>
<td>0.04 ± 0$^d$</td>
</tr>
<tr>
<td>Field cultivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First harvest</td>
<td>0.40 ± 0.05$^a$</td>
<td>0.09 ± 0.01$^c$</td>
</tr>
<tr>
<td>Second harvest</td>
<td>0.44 ± 0.10$^b$</td>
<td>0.14 ± 0.02$^a$</td>
</tr>
<tr>
<td>Third harvest</td>
<td>0.11 ± 0.04$^d$</td>
<td>0.12 ± 0.03$^b$</td>
</tr>
</tbody>
</table>

$\dagger$ Each value represents mean ± standard deviation of three replicate sublots. Within each column, means with different letters are significantly different ($p < 0.05$).

### 2.7. Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of triplicates from three independent experiments and analyzed by the SAS software package Version 9.0 (SAS Institute, Inc., Cary, NC, USA). Analyses of variance were performed using ANOVA procedures. Significant differences between means were determined using Duncan’s multiple-range test.

### 3. Results and discussion

#### 3.1. Dry mass yields and moisture contents of peanut top parts

When peanut kernels of Tainan 9 were respectively aquatic- and field-cultivated for 30 days and the top parts of peanut plants were harvested at a 10-day interval for three times, photographs of the harvested top parts are shown in Fig. 1A. Apparently, sizes of the harvested top parts decreased in order with time of harvest. Their dry mass weights of the separated leaves and stems also decreased in order with time of harvest (Table 1). In comparison, dry mass weights of the harvested leaves were higher than weights of the stems. As affected by time of harvest, yields of the third harvested leaves were much lower than those of the first and second harvests. As affected by cultivation practice, dry mass yields of the field-cultivated leaves were higher than those of aquatic-cultivated leaves. Since the medium solution was not renewed except RO water was added to keep constant volume during aquatic cultivation, the significantly lower dry mass yields observed in the third harvest may attribute to depletion of some of the original nutrients. For field cultivation, the dry mass yields of leaves for the first and second harvests were close to each other and decreased significantly for the third harvest. This might be relevant to depletion of soil nutrients or requirement of a longer cultivation period. Moisture contents of the harvested leaves and stems varied with cultivation practice and time of harvest (Table 1). In comparison, moisture contents of the first-harvest of aquatic-cultivated leaves and stems were higher than those of other harvested leaves and stems.

#### 3.2. Total phenolics contents and identification

Total phenolics contents of the 80% methanol extracts of the harvested peanut leaves and stems are shown in Fig. 2A. Contents of the aquatic-cultivated leaves and stems increased significantly from the first to the second harvest and decreased in the third harvest. For field cultivation, contents of the first-harvest leaves were about the same level as the second harvest and increased significantly for the third harvest. For the stems of field cultivation, their contents changed limitedly. In comparison among the harvested leaves, the highest phenolics content was achieved in the second harvest of aquatic cultivation and the third harvest of field cultivation. The observed higher biosynthesized quantities of total phenolics after first harvest might relate to cut-to-harvest as a physical wounding of the plants. Cut-to-harvest may create an external stress to stimulate biosynthesis of phenolic compounds as the secondary metabolites (Dixon et al., 2005; Vogt et al., 1994). Wounding-induced biosynthesis of the cell wall-bound phenolic esters, chlorogenic acid, alkyl ferulate esters and precursors for the synthesis of lignin, suberin and other polyphenolic barriers have been reported (Bernards & Lewis, 1992; Hahlbrock & Scheel, 1989). Abiotic stresses such as physical wounding (Chung et al., 2003; Smith, 1982), feeding by herbivorous insects (Smith, 1982), nutritional stress (Dixon & Paiva, 1995) and UV irradiation from sunlight (Chung et al., 2003) in elicitation of phenylpropanoid biosynthesis by activation of the stress-induced phenylpropanoid biosynthetic enzymes have been demonstrated.

When the 80% methanol extracts of the harvested leaves and stems were subjected to reverse phase HPLC analysis with a C18 column, the chromatograms varied quantitatively rather than qualitatively among the test samples (Fig. 3). Peak 1 and peak 2 were respectively identified as epicatechin and caffeic acid mainly based on matches of their HPLC retention times and UV spectra with the authentic compounds (Fig. 4). In addition to epicatechin and caffeic acid, other HPLC-detected peaks remain unidentified (Fig. 3). The contents of epicatechin and caffeic acid expressed as mg/dry mass of the harvested leaves are summarized in Table 2. Trend of changes of both contents as affected by time of harvest were similar to that of change of total phenolics contents (Fig. 2A). The
highest contents of epicatechin and caffeic acid were detected in the second aquatic-harvested leaves. In addition to cut-to-harvest as a physical wounding in stimulation of biosynthesis of the epicatechin and caffeic acid is likely. Physical wounding was related to the transcription factors that regulated genes expression of phenylpropanoid biosynthesis (Weisshaar & Jenkins, 1998). Stress-induced phenylpropanoid biosynthesis of caffeic acid is derived from cinnamic acid through phenylalanine by the action of phenylalanine ammonia-lyase (PAL) via a series of hydroxylation, methylation and dehydration reaction (Dixon & Paiva, 1995). As compared, epicatechin and caffeic acid contents in each time of harvest were higher for the aquatic cultivation than contents for the field cultivation. This reveals that aquatic-cultivated peanut plants might grow under more environmental stresses than the field-cultivated peanuts and result in biosynthesis of higher quantity of stress-related secondary phenolics metabolites.

### 3.3. Antioxidant activities

Phenolic phytochemicals have attracted extensive attentions mainly based on their effectiveness conferring health enhancement via antioxidant and free radical scavenging potencies and consequent protection of cellular components from free radical induced damage (Jabri-Karoui, Bettaieb, Msaada, Hammami, & Marzouk, 2012). Reducing power is one of common approaches in estimation of antioxidant activity or ability (Ksouri et al., 2008; Zhong, Ma, & Shahidi, 2012). When the peanut extracts were subjected to determining of reducing power, in each harvest of the aquatic- and field-cultivation, the values for the leaves were higher than the values of stems (Fig. 2B). Among the test samples, the highest reducing power was detected for the second aquatic-harvested leaves.

As subjecting the peanut extracts to another approach of antioxidant characterization based on scavenging of ABTS free radical cation (ABTS•⁺) (Madhujith et al., 2006), in each harvest of the aquatic- and field-cultivation, the values for the leaves were higher than values of the stems (Fig. 2C). The highest TEAC values were detected for the second aquatic-harvested leaves and the third field-harvested leaves. For the field-cultivated leaves and stems, both TEAC values increased significantly with time of multiple harvests. For the aquatic-cultivated leaves, reducing powers (Fig. 2B), TEAC values (Fig. 2C) and total phenolics contents (Fig. 2A) changed with correlatively.

As further compared, the reducing powers and TEAC values of leaves were also closely related to contents of epicatechin and caffeic acid (Table 2 and Fig. 3). For both aquatic- and field-cultivations, higher contents of total phenolics along with higher antioxidant activities were detected in the second-harvested leaves and stems. This may attribute to the effect of cut-to-harvest as an external wounding of stress in biosynthesis stimulation of the phenolic antioxidants. Nevertheless, the real mechanisms are sophisticated and deserve further research attention. The observed increases of antioxidant activities may also relate to combined effects of environmental temperature changes, physical wounding, nutritional stress and others. Nutritional stresses such as low iron level has been noticed to increase biosynthesis of phenolic acids, low phosphate to induce more anthocyanins biosynthesis and low nitrogen to induce less biosynthesis of flavonoids and isoflavonoids (Dixon & Paiva, 1995).

### 3.4. Antiglycative activities characterization

For antiglycative activity assessment, each of the peanut extracts was introduced to the reactants of BSA and fructose and followed by incubation at 50 °C for 24 h for fluorescence determination (Fig. 2D). For each harvest, the inhibitory activities of the leaves were higher than the stems. It is noteworthy that all leaf extracts exhibited potent inhibitory activities against AGEs formation and the activities varied insignificantly among the test leaf extracts. All leaf extracts exhibited higher activities than that of 3 mM of AG as a positive control determined concurrently. As further subjection of the reacted solutions to SDS–PAGE analysis, a band with ca. 97 kDa molecular weight of a glycated protein (Wang et al., 2011; Zhang, Wang, & Dong, 2011) (indicated with an arrow that all leaf extracts exhibited potent inhibitory activities against AGES formation and the activities varied insignificantly among the test leaf extracts. All leaf extracts exhibited higher activities than that of 3 mM of AG as a positive control determined concurrently. As further subjection of the reacted solutions to SDS–PAGE analysis, a band with ca. 97 kDa molecular weight of a glycated protein (Wang et al., 2011; Zhang, Wang, & Dong, 2011) (indicated with an arrow in Fig. 5) was detected in the reaction of BSA and fructose (lane II). When the peanut leaf extracts were introduced for reaction (lanes A-1 to F-3), formation of the glycated protein was inhibited effectively. In this study, richness of phenolics contents especially caffeic acid and epicatechin in the peanut leaves and consequent antioxidant and antiglycative activities have been demonstrated (Table 2 and Fig. 2A). This was in agreement with the observations that phenolic compounds exhibit the major role of antiglycative agents in various plant conditions.
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