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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

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its derivatives have been detected in mulberry leaves of Tunisian *Morus* species (Thabti, Elfalleh, Hannachi, Ferchichi, & Campos, 2012). Caffeic acid is a major compound in the leaves of sugarcane (*Saccharum officinarim* 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 hypertention (angiotensin I converting) (Oboh et al., 2012). The phenolic compounds, including caffeic, ferulic, vanillic, *p*-hydroxybenzoic and protocatechuic 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 antioxidative 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 antiglycative 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. hypogaea* 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 antiglycative 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 antiglycative 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