

## PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF PEANUT'S SKIN, HULL, RAW KERNEL AND ROASTED KERNEL FLOUR

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### Abstract

In this study, phenolic compounds and antioxidant properties of peanut's skin, hull, raw kernel and roasted kernel flour (RKF) were evaluated. Total phenolic contents (TPC) and individual phenolic compounds were determined using Folin-Ciocalteu and high performance liquid chromatographic methods, respectively. Antioxidant activity was measured utilizing 2, 2-diphenyl-1-1 picrylhydrazyl (DPPH) radical scavenging capacity and inhibition of linoleic acid peroxidation assays. Results of the study showed that antioxidant activity and phenolic compounds of peanut skin were highest followed by that of peanut hull, roasted kernel flour (RKF) and raw kernel. Roasting of peanut kernels at 160°C for 10 min did not affect the overall antioxidant activity and phenolic compounds of RKF. In the present work, a good correlation was recorded between TPC and radical scavenging capacity ( $r^2 = 0.8436$ ) as well as TPC versus % inhibition of linoleic acid peroxidation ( $r^2 = 0.6535$ ).

### Introduction

Antioxidants are often added to foods to prevent the formation of free radicals and slow down the oxidation process by inhibiting the initiation, propagation steps, leading to the termination of reaction (Shahidi & Amarowicz, 1994). Antioxidant can be used to retard formation of reactive oxygen species and help to maintain nutritional quality and shelf-life of foods (Jadhav *et al.*, 1996). Several synthetic antioxidants, namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG), and ethyl protocatechuate (EP) have been used in foods to prevent oxidation. However, the use of synthetic antioxidants in foods are restricted by legislation and discouraged due to their perceived toxicity and carcinogenicity (Madavi & Salunkhe, 1995). Therefore, extracts of herbs, vegetables, fruits, cereals, nuts and other plant products rich in phenolics, are of increasing interest for the food industry as natural antioxidant ingredients (Sang *et al.*, 2002).

Peanut (*Arachis hypogaea* L.) is one of the major oilseed crops of the world. It is also the important sources of food protein in many countries. Peanuts can be consumed as raw, pureed, roasted or mixed with other foods or in different processed forms. Recently, peanuts have gained much attention as functional food (Francisco & Resurreccion, 2008). Recent studies suggest that peanuts consumption might reduce the risk of heart diseases by lowering serum low-density lipoprotein (LDL)-cholesterol level and reduce the risk in the development of type II diabetes (Fraser *et al.*, 1992). The health benefits of peanuts have been attributed to the presence of minerals and vitamins, fatty acids, fiber and bioactive compounds (Griel *et al.*, 2004).

Peanut comprises of skin, hull and kernel (seed). Peanut skin and hull are by-products of peanut processing industry. These are often considered as agrowaste but some time used for animal feedstuffs and fertilizers. However, peanut skin also contains considerable amount of phenolics and other health-promoting compounds and thus can be explored for functional food applications (Yu *et al.*, 2005). Peanut hulls were also found to exhibit appreciable antioxidant activity and antimutagenic effect (Duh & Yen, 1997). Peanut kernels are used to make peanut butter, snack and cooking oil. Roasted kernel flour (RKF) is formed after the partial extraction of oil from roasted peanuts and commonly used as food ingredients in peanut industry. Roasted peanut kernels were found to be

rich in antioxidants as that of blackberries and strawberries (Talcott *et al.*, 2005).

Roasting was found to increase the antioxidant capacity of intact peanuts due to the formation of Maillard reaction products (Talcott *et al.*, 2005). The phenolics present in the roasted peanuts may vary between commercial peanut cultivars. Despite the large available data on the antioxidant properties of peanuts, additional information are needed to ascertain the specific compounds that contribute to the antioxidant activity and to evaluate the application of peanut parts as natural antioxidant source. The main objective of the present study was to investigate and compare the phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour.

### Materials and Methods

**Reagents and Samples:** Raw, fresh in-shell peanut pods (Virginia spreading type) were obtained from an Agricultural Farm in Nay-Pyi-Taw Township, Myanmar. Three different peanut samples were harvested. Trifluoroacetic acid (TFA), phenolic acids standards (hydroxybenzoic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, gallic), flavonoids (epicatechin, dihydroquercetin, luteolin, kaempferol) and stilbene (trans-resveratrol), butylatedhydroxyanisole (BHA),  $\alpha$ -Tocopherol, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) were from Sigma Chemical Company (St. Louis, MO, USA), thiobarbituric acid (TBA), linoleic acid, ammonium thiocyanate, ferrous chloride, Tween 20, 2,4,6-triphenyl-s-triazine, ferric chloride were obtained from Fisher Scientific (Ottawa, ON, Canada). All other reagents were of analytical grade or higher purity and obtained from Fisher Scientific.

**Sample Preparation:** Raw peanuts pods (3 kg) were dried at room temperature ( $28 \pm 1^\circ\text{C}$ ) for 2 days. After drying, pods were manually shelled and the skins were removed from the raw peanut kernels. The hulls (shells) and skins were ground using a commercial grinder (Pensonic, Malaysia) and stored at  $-20^\circ\text{C}$  for further analysis. For preparation of roasted peanut flour, approximately 1kg raw kernels were heated at  $160^\circ\text{C}$  for 10 min in an oven (Mettler, Germany) equipped with an air circulation system. After cooling at room temperature, roasted peanut kernels were then ground and sieved to get fine powder. Raw kernels were also ground under the same condition. Both samples were kept at  $-20^\circ\text{C}$  until further analysis.

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**Extraction of Antioxidant Components:** Ground hull and skin (10 g) were extracted with 100 mL methanol by shaking on a water bath for 2 hours at room temperature ( $28 \pm 1^\circ\text{C}$ ). The ground raw and roasted kernel flour (RKF) were defatted first with hexane (10% w/v) using a soxhlet extraction unit for 8 h. The defatted samples were then air-dried and extracted with methanol (100 mL) using a water bath shaker (Protect, Model 903, Malaysia). All suspensions were then filtered through a Whatman No.1 filter paper and the residues re-extracted twice, each time with additional 100 mL of the same solvent. The filtrates were combined and the solvent evaporated under reduced pressure using a rotary evaporator (Eyela, Model N-1000) at  $40^\circ\text{C}$ . The methanolic extracts were used for the determination of total phenolics and antioxidant activity.

**Total Phenolics Contents (TPC) Determination:** A modified Folin-Ciocalteu procedure as described by Jayaprakasha *et al.*, (2001) was used for the determination of total phenolic contents. Samples (0.4 mL) were mixed with 2.0 mL of the Folin-Ciocalteu reagent (diluted 10 times), and the reaction was terminated using 1.6 mL of 7.5% sodium carbonate. After 30 min incubation at room temperature ( $28 \pm 1^\circ\text{C}$ ), the absorbance was read at 750 nm using a spectrophotometer (Shimadzu, Japan). The standard curve was prepared using gallic acid standard solutions of known concentrations, and the results were expressed as mg gallic acid equivalent/g sample.

**Free Radical Scavenging Assay (DPPH):** The antioxidant capacity of peanut's extracts derived from skin, hull, raw and RKF was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method of Brand-Williams *et al.* (1995) with slight modifications. Methanol extract (0.5 mL) of sample at various concentrations were added to 2.5 mL of freshly prepared DPPH solution (25 mg/L). The mixture was incubated for 30 min at room temperature and the decrease in absorbance at the end of incubation period was measured using a spectrophotometer (Shimadzu, Japan) at 515 nm. Pure methanol was used as blank. BHA and  $\alpha$ -tocopherol were employed as reference antioxidants for this assay. The

percent DPPH scavenged by each samples were calculated by the following equation;

$$\% \text{ DPPH scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

where,  $A_0$  = absorbance of DPPH solution without sample/standard solution, and  $A_1$  = absorbance of the sample/standard solution at 30 min reaction. Measurements were taken in triplicate and the data was expressed as mean  $\pm$  standard deviation (SD).

#### Antioxidant Activity in Linoleic Acid Peroxidation System:

Methanol extracts of peanut hull, skin, raw and RPF were tested for its antioxidant activity by measuring the inhibition of linoleic acid peroxidation following a previously reported method of Yen & Hsieh (1998). Test sample (0.5 mL) dissolved in absolute ethanol was mixed with 2.5 mL of linoleic acid emulsion (0.02M, pH 7.0) in phosphate buffer. This emulsion was prepared by mixing and homogenizing 0.280g of linoleic acid, 0.280 g Tween 20 as emulsifier and 50 mL of phosphate buffer solution. The resulting reaction mixture was then subjected to incubation at  $37^\circ\text{C}$  for 96 h and exactly 0.1 mL portion from the mixture was withdrawn at different intervals during incubation. The magnitude of linoleic acid oxidation was determined by the peroxide value following a colorimetric method as described by Yen *et al.*, (2000). Briefly, to 0.1 mL sample solution, 4.7 mL of ethanol (75%), 0.1 mL of ammonium thiocyanate (30%), and ferrous chloride (0.1mL, 0.02M in 3.5 % HCL) were added sequentially. After the mixture had left for 3 min, the absorbance of the resulting reaction mixture was read at 500 nm using a spectrophotometer (Shimadzu, Japan). The degree of oxidation was measured after every 24 h until a day after the absorbance of the control reached its maximum. A control with linoleic acid but without the sample extract was performed simultaneously. Besides, BHA,  $\alpha$ -tocopherol and quercetin were used as positive controls. The percent inhibition of linoleic acid peroxidation was calculated as the following equation:

$$\text{Inhibition of linoleic acid peroxidation (\%)} = \left( 1 - \frac{\text{Absorbance at 500 nm in the presence of sample 96h}}{\text{Absorbance at 500 nm in the absence of sample 96h}} \right) \times 100$$

**Determination of Individual Phenolic Compounds by HPLC:** The identification of the individual phenolic including phenolic acids, flavonoids and resveratrol were carried out using a Waters HPLC system. The hydrolysis was carried out using the method of Wang *et al.*, (2008) with slight modifications. Peanut skin (0.2 g), hull, raw kernel and RKF (0.5 g) were dissolved in 10 mL 80% methanol and 1 mL of 1.2 M hydrochloric acid and hydrolyzed by incubating in a water bath at  $80^\circ\text{C}$  for 2 h. After hydrolysis, the mixture was cooled and centrifuged at  $1200 \times g$  for 5 min and the supernatant recovered was filtered using a  $0.45\text{-}\mu\text{m}$  nylon membrane filter prior to HPLC analysis. The phenolic standards solutions were prepared by dissolving respective pure compounds in absolute methanol.

A Waters HPLC system with Waters 2487 Dual Wavelength Absorbance Detector, Waters 600 Pump and controlled by Waters Empower2 software (Waters, Milford, MA) was used. The separation of the phenolics was carried out on Waters reverse-phase (RP) Symmetry  $\text{C}_{18}$  column ( $150 \times 3.9$  mm,  $5\text{-}\mu\text{m}$ ) at room temperature ( $28 \pm 1^\circ\text{C}$ ). The mobile phase consisted of TFA in deionized water (pH 2.5) as solvent A and absolute methanol (99.99%) as solvent B. The gradient

conditions used were as follows: 100-50% solvent A (0-20 min), 50-40% solvent A (20- 30 min) and 40-100% solvent A (30 - 40 min). The flow rate of mobile phase was set at 1.0 mL/min, and a  $20\text{-}\mu\text{L}$  sample volume was injected. The detection of phenolic compounds was monitored at 280 nm (Yurttas *et al.*, 2000). Identification of the unknown phenolics was based on matching their retention times with those of pure standards of phenolics. Peak area was used for quantification using external standard calibration curves and the amounts were expressed as  $\mu\text{g/g}$  sample.

**Statistical analysis:** All analyses were carried out in triplicates and data expressed as means  $\pm$  standard deviations. One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess significant differences between means ( $p < 0.05$ ) using SPSS version 16.0. Correlations between total phenolic compounds and antioxidant activity were done utilizing bivariate Pearson procedure.

#### Results and Discussion

**Total Phenolic Contents (TPC):** In general, the higher amount of polyphenolic compounds present in the outer layers of plants

parts such as shell, hull and peel is to protect the inner materials. Several plant phenolics have been identified to exhibit various degree of antioxidant activity (Scalbert *et al.*, 2005). In the present work, TPC was determined using Folin-Ciocalteu (FCR) method which is considered as one of the best methods for the determination of TPC (Sultana *et al.*, 2008).

Results of the present study showed that TPC of peanut skin (91.74 mg GAE/g) was significantly ( $p < 0.05$ ) higher than that of peanut hull (27.59 mg GAE/g) followed by RKF (1.17 mg GAE /g), and raw kernels (0.92 mg GAE/g) (Table 1). The higher TPC of peanut skin may be attributed to the presence of phenolic compounds such as proanthocyanidins (Yvonne *et al.*, 2007). The TPC content of peanut skin found in this study was comparable to that reported by Wang *et al.*, (2007). However, Nepote *et al.*, (2005) reported that TPC in peanut skin was 118 mg/g dry skin. Yu *et al.*, (2005) also found that one gram dry peanut skin contained 90-125 mg of total phenolics and the skin removal methods (direct peeling, blanching, and roasting) and types of solvents used for extraction significantly affected the total phenolics. Similarly, TPC of methanol extract of peanut hull from various cultivars was 4.2-10.2 mg /g hull (Duh and Yen, 1995) which was lower than that found in this study.

Interestingly, RKF showed higher TPC compared to that of raw kernel, although they were not significantly different ( $p < 0.05$ ). Similar result was reported by Talcott *et al.*, (2005). The authors showed that phenolic contents of 'Georgia Green' cultivar increased from 0.913 mg/g (raw kernels) to 0.949 mg/g (roasted kernels), when the kernels were roasted at 170°C for 10 min. Naturally occurring polyphenols serve as primary sources of antioxidants. During food processing (heating or boiling), certain chemical reactions may occur among the food components and lead to generate secondary antioxidants compounds such as Maillard Reaction Products (MRPs) (Dittrich *et al.*, 2003). In peanut roasting, soluble proteins and amino acids are changed as a result of moisture losses and form Maillard derivatives, including pyrroles and furans which may contribute to the increased in total phenolic compounds of roasted samples (Yanagimoto *et al.*, 2002).

**Free Radical Scavenging Activity (DPPH):** Phenolic compounds found in plants are powerful antioxidants due to their ability to donate hydrogen or electron and to form stable radical intermediates (Scalbert *et al.*, 2005). DPPH is a stable nitrogen centered free radical, which is used to evaluate antioxidant activity because of its short span of radical quenching capacities (Brand- Williams *et al.*, 1995). DPPH radical with deep violet color gives intensive absorption within 515-528 nm range. This test is based on the ability of the DPPH radical to react hydrogen donor species, mainly phenolics. Upon receiving proton from extract constituents, DPPH radical loses its color and becomes yellow. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic compounds increases, their DPPH scavenging activity also increases, thus correlating directly to the extent of antioxidant efficacy of a typical plant material (Roginsky & Lissi, 2005).

In this study, the radical scavenging activity of the each sample was compared at a concentration of 500 µg/mL. Results of the study showed that the order of free radical scavenging activity of extracts from peanut parts was as follows; skin > hull > RKF > raw kernel (Table 1). Both peanut skin and hull, although considered as agro wastes, exhibited very good free radical scavenging activity. The extract from the peanut skin exhibited the highest antioxidant activity (89.97%) that was comparable with that of both BHA (90.50%) and  $\alpha$ -tocopherol (88.43%). In agreement with our results, Wang *et al.*, (2007) revealed that peanut skin extracts showed higher scavenging

activity than that of BHA, synthetic antioxidant at a concentration range of 10-500 µg/mL.

In this study, it was found that radical scavenging activity of peanut hull (61.09%) was significantly ( $p < 0.05$ ) higher than that of raw kernel (11.74%) and RKF (16.15%). Hwang *et al.*, (2001) reported that roasted peanuts (180±2°C for 60 min) exhibited higher scavenging activity as measured by DPPH method. However, in the present study, peanut kernels roasted at 160°C for 10 min slightly increased the antioxidant activity that was not significantly ( $p > 0.05$ ) different from that of raw kernel. This was probably due to the short heating time used in the study. Lee *et al.*, (2003) showed that simple heat treatment could not break the bound-form of phenolic compounds from rice hull while far-infrared treatment could influence the composition of phenolics. In addition, it has been indicated that effectiveness of processing step to liberate antioxidant compounds from plants may vary depending on species (Jeong *et al.*, 2004).

#### **Antioxidant Activity in Linoleic Acid Peroxidation System:**

Antioxidant activity of peanut skin, hull, raw kernel and RKF was determined in terms of inhibition of linoleic acid peroxidation (Table 1). Linoleic acid is a polyunsaturated fatty acid where, upon oxidation process, peroxides are formed which oxidizes  $\text{Fe}^{+2}$  to  $\text{Fe}^{+3}$ , the later forms complex with thiocyanate ion. The intensity of this colored complex is measured at 500 nm. The higher the absorbance of the sample indicated the lower the antioxidant activity.

According to the results (Table 1), at a concentration of 500 µg/mL, peanut skin and hull extracts exhibited higher inhibition of peroxidation in linoleic acid system (82.10% and 79.85%, respectively), than that of raw kernel (26.57%) and RKF (29.31%). However, the activities were significantly ( $p < 0.05$ ) lower than that of synthetic antioxidant, BHA (88.85%), but higher than quercetin (58.73%) and comparable to that of  $\alpha$ -tocopherol (82.51%). Saha *et al.*, (2004) reported that methanol extracts of Malaysian medicinal plants showed higher antioxidant activity than that of quercetin but lower than that of BHA as determined by linoleic acid peroxidation system. Sultana *et al.*, (2008) also reported that phenolic contents of various agricultural wastes exhibited potential antioxidant activity against linoleic acid peroxidation system. Hwang *et al.*, (2001) pointed that extracts of roasted and defatted legume kernels displayed the most remarkable antioxidative activity on a linoleic acid emulsion system. Also Siddhuraju and Becker (2007) found that dry heated cowpea seed extracts exhibited potential antioxidative efficiency in a linoleic acid-thiocyanate system. However, in contrast to our present results, there was no significant difference ( $p < 0.05$ ) observed between the antioxidative activities of raw kernels and RKF. It might be due to the different heating treatments and types of samples used in the study.

#### **Correlation between TPC and Antioxidant Activity:**

The correlation coefficients showing the relationship between TPC, DPPH assay and linoleic acid peroxidation system are presented in Figs. 1 and 2. Antioxidant activity of phenolic compounds is mostly associated with their redox properties which allow them to act as antioxidative agents (Siddhuraju & Becker, 2003). Figure 1 shows a good correlation coefficient ( $r^2 = 0.8436$ ,  $p < 0.01$ ) between TPC and DPPH scavenging activity. On the other hand, a moderate correlation ( $r^2 = 0.6535$ ,  $p < 0.05$ ) was seen between TPC and percent inhibition of lipid peroxidation (Fig. 2). In previous studies, good correlation between the TPC and DPPH free radical scavenging capacity was reported for different barley varieties ( $r^2 = 0.799^{**}$ ) (Zhao *et al.*, 2008) and sweet potato leaves extracts ( $r^2 = 0.770^{**}$ ) (Xu *et al.*, 2010). Similarly, Orak, (2007) reported a higher correlation between percent

inhibition of lipid peroxidation and concentration of total phenols in selected red grape cultivars ( $r^2 = 0.756^{**}$ ). Our present investigation reveal that TPC is mainly responsible for

DPPH free radical scavenging capacity and percent inhibition of linoleic acid peroxidation of the tested samples.

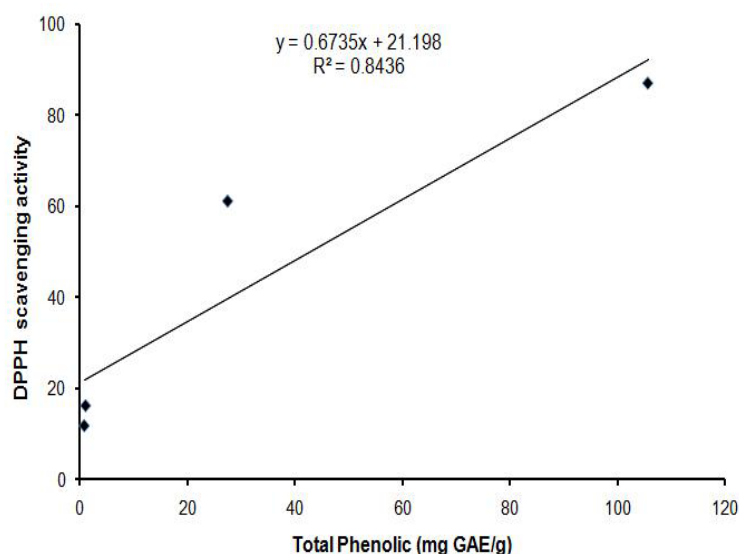


Fig. 1. Correlation between TPC and DPPH radical scavenging activity.

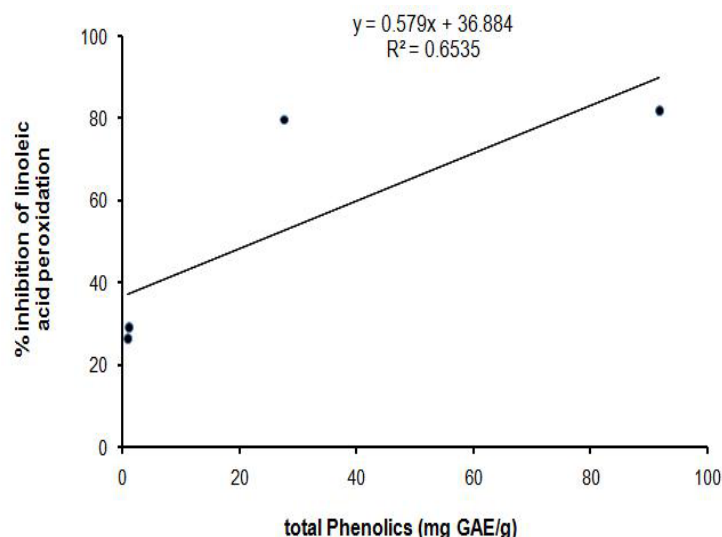


Fig. 2. Correlation between TPC and % inhibition of linoleic acid peroxidation.

**Table 1. Total phenolic contents (TPC), DPPH radical scavenging activity, percent inhibition of linoleic acid peroxidation of peanut skin, hull, raw kernel, and roasted kernel flour.**

Samples	TPC (mg GAE/g)	DPPH Radical scavenging activity	% Inhibition of linoleic acid peroxidation
Skin	91.74 ± 5.08 <sup>a</sup>	89.97 ± 1.87 <sup>a</sup>	82.10 ± 1.55 <sup>b</sup>
Hull	27.59 ± 2.82 <sup>b</sup>	61.09 ± 4.15 <sup>b</sup>	79.85 ± 1.82 <sup>b</sup>
R K F	1.17 ± 0.24 <sup>c</sup>	16.15 ± 3.46 <sup>c</sup>	29.32 ± 1.96 <sup>d</sup>
Raw kernel	0.92 ± 0.16 <sup>c</sup>	11.74 ± 0.69 <sup>c</sup>	26.57 ± 2.71 <sup>d</sup>
BHA	NA	90.50 ± 1.84 <sup>a</sup>	88.85 ± 0.55 <sup>a</sup>
α-tocopherol	NA	88.43 ± 1.48 <sup>a</sup>	82.51 ± 0.83 <sup>b</sup>
Quercetin	NA	NA	58.73 ± 2.42 <sup>c</sup>

Values are mean ± standard deviation for three replicates. Mean values in the same column followed by different superscript letters are significantly different ( $p < 0.05$ ) among different parts of peanut. NA- not applicable.

**Individual Phenolics Composition:** Plant phenolics include simple phenolic acids, stilbenes, flavonoids and other group of polyphenolic compounds. Flavonoids can be divided into six major groups including flavones, flavonols, flavanones, anthocyanidins, catechins and isoflavones based on the presence of heterocyclic C ring (Ross & Kasum, 2002). Phenolic acids (*p*-hydroxybenzoic, chlorogenic, ferulic, caffeic, *p*-coumaric, gallic), flavonoids (epicatechin, quercetin, daidzein, kaempferol and luteolin) and stilbene (resveratrol) were selected for analysis based on the fact that they are commonly found in oil seeds such as peanuts.

Individual phenolic composition of peanut's hull, skin, raw kernel and RKF was analysed by HPLC and the results presented in Table 2. In the study, numerous phenolics were detected in peanut skin (Fig. 4a). However, all of those compounds could not be quantified because of their very low concentrations and the peaks seem to be suppressed by major procyanidin compounds as peanut skin contains a complex series of procyanidin oligomers (Francisco & Resurreccion, 2009). Results of the study showed that *p*-coumaric acid was significantly ( $p < 0.05$ ) highest in RKF (40.67 µg/g) and raw kernels (35 µg/g) and lower in peanut skin (28.80 µg/g). Similarly, the amount of *p*-hydroxybenzoic acid was significantly ( $p < 0.05$ ) higher in RKF (169 µg/g), raw kernel (157 µg/g) and peanut hull (146 µg/g) followed by peanut skin (13.08 µg/g). Previous studies reported that predominant phenolics present in peanut kernel were *p*-hydroxybenzoic and *p*-coumaric acid (Talcott *et al.*, 2005). After roasting, the amount of *p*-hydroxybenzoic acid and *p*-coumaric acid were slightly increased however these values were not significantly different from each other. Ferulic acid (39.82 µg/g) was detected only in peanut skin.

**Table 2. Individual phenolics ( $\mu\text{g/g}$  dry sample) composition in peanut's skin, hull, raw kernel and roasted kernel flour as measured by HPLC.**

Phenolic compound	Skin	Hull	R K F	Raw kernel
P-hydroxybenzoic acid	$13.08 \pm 1.53^b$	$146.00 \pm 34.00^a$	$169.00 \pm 12.73^a$	$157.00 \pm 8.11^a$
Chlorogenic acid	$21.95 \pm 7.35^a$	ND	$2.20 \pm 0.11^b$	$2.42 \pm 0.06^b$
<i>P</i> -coumaric acid	$58.80 \pm 4.50^a$	ND	$40.67 \pm 7.90^b$	$35.00 \pm 2.83^b$
Ferulic acid	$39.82 \pm 0.22$	ND	ND	ND
Resveratrol	$2.99 \pm 0.67^a$	$3.09 \pm 0.13^a$	$0.11 \pm 0.22^b$	$0.10 \pm 0.04^b$
Epicatechin	$159.60 \pm 11.28$	ND	ND	ND
Quercetin	$17.72 \pm 1.65^a$	ND	$13.10 \pm 2.40^b$	$12.40 \pm 1.28^b$
Luteolin	ND	$1071.50 \pm 77.00$	ND	ND
Kaempferol	ND	ND	$1.27 \pm 0.19^a$	$1.59 \pm 0.33^a$

Values are mean  $\pm$  standard deviation for three replicates. Mean values for each compound in the same row followed by different superscript letters are significantly different ( $p < 0.05$ ) among different parts of peanut. ND= not detected.

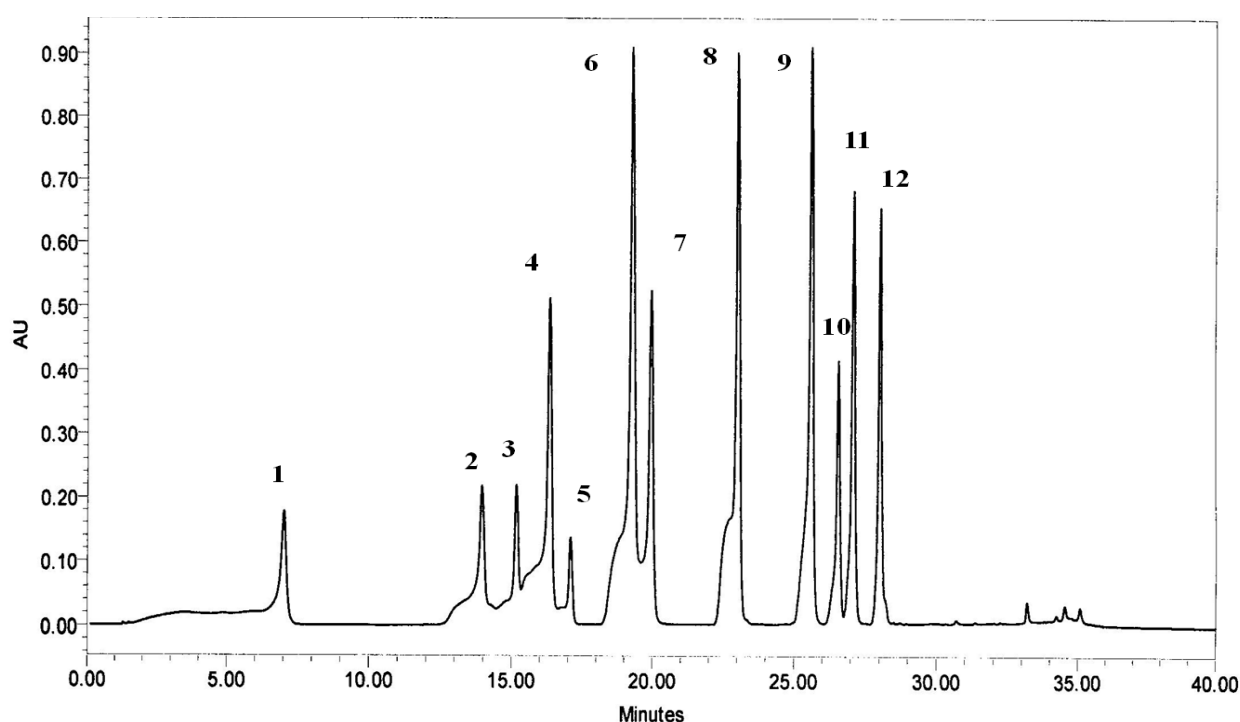


Fig. 3. HPLC chromatogram of 12 phenolic compound standards at 280 nm. Peak identification: (1) gallic acid, (2) *p*-hydroxybenzoic acid, (3) chlorogenic acid, (4) caffeic acid, (5) epicatechin, (6) *p*-coumaric acid, (7) ferulic acid, (8) resveratrol, (9) quercetin, (10) daidzin, (11) luteolin, (12) kaempferol.

Resveratrol is one of the major stilbene phytoalexins that are produced by different parts of the peanuts. Substantial amounts of resveratrol was found in the leaves, roots, and shells of peanuts but the levels noted to be lower in developing seed and seed coats of peanuts (Chung *et al.*, 2003). In this study, peanut hull was found to contain higher amount of resveratrol ( $3.09 \mu\text{g/g}$ ) which was significantly ( $p < 0.05$ ) higher than that of raw kernels ( $0.10 \mu\text{g/g}$ ) and RKF ( $0.11 \mu\text{g/g}$ ) but not significantly difference from that of peanut skin ( $2.99 \mu\text{g/g}$ ). The maturity of peanut may also affected resveratrol content where it was reported that immature peanuts have higher levels of resveratrol than that of mature peanuts (Sobolev & Cole, 1999).

As flavonoids, epicatechin ( $159.60 \mu\text{g/g}$ ) was only detected in peanut skin and this value was comparable to that ( $144.75 \mu\text{g/g}$  for Virginia type peanut skin) investigated by Francisco & Resurreccion (2009). The highest luteolin content ( $1071.50 \mu\text{g/g}$ ), on the other hand, was found in peanut hull

although the result was lower when compared with that ( $2470 \mu\text{g/g}$  hull) reported by Duh & Yen (1995). Such differences in data may be due to the different cultivars and different maturity/harvesting stages of peanuts used in the studies. The earlier studies have shown that luteolin content of peanut hulls increased with maturity (Yen *et al.*, 1993). Quercetin was reported to be the major flavonoid in peanut seed (Wang *et al.*, 2008). In the present study, significantly ( $p < 0.05$ ) higher amount of quercetin ( $17.72 \mu\text{g/g}$ ) in peanut skin was detected followed by RKF ( $13.10 \mu\text{g/g}$ ) and raw kernel ( $12.40 \mu\text{g/g}$ ). The amount of quercetin detected in our study was quite lower than that reported by Wang *et al.*, (2008). In their report, significant variation in quercetin content occurred among different seed-coat color peanut seeds. In agreement with the findings of Wang *et al.*, (2008), in our present experiment, a small amount of kaempferol was detected in RKF ( $1.27 \mu\text{g/g}$ ) and raw kernel ( $1.59 \mu\text{g/g}$ ).

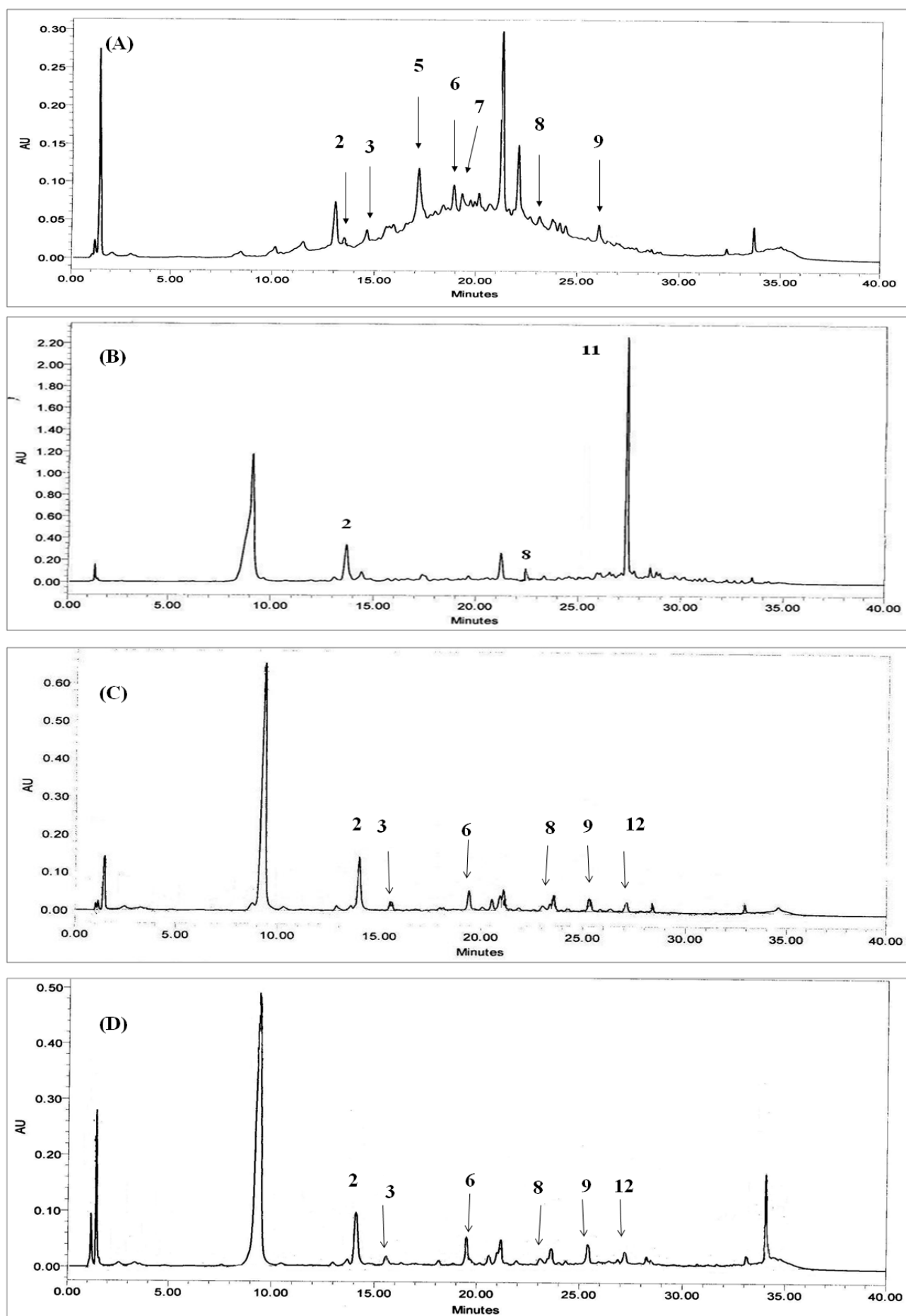


Fig. 4. Phenolic compounds detected in the peanut skin (A), peanut hull (B), raw kernel (C), and roasted kernel flour (RKF) (D). Peak identification: (2) *p*-hydroxybenzoic acid, (3) chlorogenic acid, (5) epicatechin, (6) *p*-coumaric acid, (7) ferulic acid, (8) resveratrol, (9) quercetin (11) luteolin (12) kaempferol.

## Conclusion

Among the tested samples, peanut skin contained relatively higher amount of phenolic compounds and also exhibited superior antioxidant activity, even comparable with those of synthetic antioxidants (BHA &  $\alpha$ -Tocopherol). The tested peanut components possessed varying but meaningful antioxidant activity which was correlated well to their total phenolic contents. Peanut kernel roasting at 160°C for 10 min was not effective towards enhancing the overall antioxidant activity of roasted kernel flour. Therefore, further studies are recommended to optimize the time and temperature of peanut kernel flour processing for standardizing and maximizing the antioxidant attributes of roasted kernel flour. In conclusion, peanuts processing by-products namely peanut skin and hull, being inexpensive source of natural antioxidants, could be explored as valuable ingredients for functional foods.

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