African Journal of Biotechnology Vol. 11(53), pp. 11547-11551, 3 July, 2012
Available online at http://www.academicjournals.org/AJB
DOI:10.5897/AJB11.4027
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Antioxidant capacity, nutritional and phytochemical content of peanut (*Arachis hypogaea* L.) shells and roots

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Accepted 6 June, 2012

This study evaluated antioxidant capacity, nutritional and phytochemical content of shells and roots of peanut (*Arachis Hypogaea* L.) for potential utilizations. Total dietary fiber, protein, ash and alkaloid per 100 g dry weight samples ranged from 58.8 to 78.2 g, 5.8 to 6.1 g, 6.6 to 21.7 g and 5 to 23.8 g, respectively. While total phenolic, total saponins and phytic acid per 100 g dry weight samples ranged from 175 to 431 mg tannic acid equivalent, 10.8 to 23.4 mg and 108 to 159 mg, respectively. Peanut roots exhibited the highest total dietary fiber, phytochemical content and the highest α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity.

Key words: *Arachis hypogaea* L, peanut shell, peanut root, total dietary fiber, phytochemical, antioxidant.

INTRODUCTION

Peanut is an important oil seed and food crop in the world. The peanut plant, exclusive of the peanut fruit, accounts for approximately 40% of the peanut biomass. Global peanut production has increased to 35.88 million metric tons in year 2011 (USDA, 2012) which led to concern on agricultural waste management. One of the best alternatives in curbing agricultural waste problems is to transform agricultural waste to underutilized material (Dongmeza et al., 2009). Thus, compositional analysis on agricultural waste is essential, in order to identify its potential applications. Most plant-based agricultural wastes are a rich source of dietary fibre and phyto-chemical which may be further utilized as functional ingredients. However, one major concern of utilizing agricultural waste as a feed is the presence of excessive phytochemical that may exert anti-nutritional or anti-physiological effects in animals (D'Mello, 2000).

Researchers have investigated variation in antioxidant activities of peanut shell extract as affected by pre-treatments (Lee et al., 2006). Chung et al. (2003) reported that peanut plants contain resveratrol, a kind of natural products with antioxidant and anti-cancer properties. However, information on nutritional and phytochemical properties of peanut shells as well as peanut roots is rather scant. This study evaluates antioxidant capacity, nutritional composition and the content of phytochemicals such as phytic acid, saponins and alkaloid, total phenolic of peanut by-products, which may provide insight on the utilization of peanut shells and roots.

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Abbreviations: PRoot, Peanut roots of Menglembu variety; **Menglembu PS,** peanut shells of Menglembu variety; **Shandong PS,** peanut shells of Shandong variety; **TAE,** tannic acid equivalent.

MATERIALS AND METHODS

Sample preparation

Varieties of peanut Shandong and Menglembu are commonly found in South East Asia. The Menglembu variety has small and round seeds and light-color shell whereas the Shandong variety, has plump grain size and elongated shape. Roots of Menglembu variety

(PRoot) and shell of both Menglembu and Shandong varieties (Menglembu PS and Shandong PS) were provided by Thong Thye Groundnut Factory Sdn Bhd near Sungai Siput, Perak, Malaysia. The peanut roots and shells were sieved to remove other debris and dirt. Then, the samples were ground and sieved through 0.5 mm, and stored in air-tight containers at 4°C before further analysis.

Nutritional content determination

Moisture and ash content of ground samples were determined according to standard AOAC method (1995). Total dietary fiber was determined by enzymatic-gravimetric method 985.29 (AOAC, 1995) using the fiber assay kit (Megazyme K-TDFR, Wicklow, Ireland) (Prosky et al., 1992). Protein content was determined by the Kjedahl method. Total dietary fiber, protein and ash content were expressed in g/100 g sample, dry weight (DW)

Phytochemical properties determination

Methanol extraction

One gram of ground sample was mixed with 10 volumes of methanol and maintained overnight at room temperature. Sample extract obtained by vacuum filtration and vacuum-evaporation (Büchi, Switzerland) at 40°C, was used for the determination of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity and total phenolic content.

Antioxidant capacity determination

Antioxidant capacity was determined based on DPPH radical scavenging activity (Brand-Williams et al., 1995). Different extract concentrations (0.5 to 3 mg/ml) were mixed with DPPH solution (60 µM), shaken vigorously and kept in the dark for 30 min. The absorbance of the sample mixture was measured at 517 nm against methanol as blank. The radical scavenging activities were calculated by subtracting A_{sample} from $A_{control}$ (absorbance of a mixture of DPPH and methanol), using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{Control} - A_{Sample})/A_{Control}] \times 100\%$$

For better comparison of the DPPH scavenging activity, the results obtained from the radical scavenging experiments were also expressed as IC_{50} values. IC_{50} is the extract concentration at which DPPH radicals were reduced by 50% and calculated from the linear regression analysis.

Total phenolic determination

The method described by Kähkönen et al. (1999) was modified. Aliquots of methanol containing 0.6 mg sample extract were mixed with diluted Folin- Ciocalteu reagent and sodium carbonate solution (7.5% w/v) in a ratio of 1:5:4; and kept in the dark for 30 min. The absorbance of the mixture was measured at 765 nm. A calibration curve was established with different concentration of tannic acid for estimating the total phenolic content in the samples. Concentration of total phenolic was expressed as mg of tannic acid equivalent (TAE)/g DW.

Total saponins determination

Saponins extraction and recovery from pre-weighed samples were done based on method of Makkar et al. (2007). A freeze-dried saponins extract was dissolved in aqueous methanol (80%) and reacted with vanillin reagent (8%) and sulphuric acid (72%) in a ratio of 1:1:10 at 60°C for 10 min before the absorbance was measured at 544 nm against a reagent blank (Hiai et al., 1976). A standard curve was established by using standard diosgenin solution (0.1 to 0.5 mg/ml) for estimating total saponins content in the sample, which was expressed as mg/100 g DW.

Phytic acid determination

Phytic acid assay was carried out following the procedure of Wheeler and Ferrel (1971). Phytic acid in ground sample was extracted with trichloroacetic acid and precipitated as ferric salt. Assuming a constant 4 Fe/6 P molecular ratios in the precipitate, phytic acid as phytate content was calculated based on iron content in the precipitate (Haug and Lantzsch, 1983) and expressed as mg/100 g DW.

Alkaloid determination

Alkaloid content was determined gravimetrically (Sreevidya and Mehrotra, 2003). Five grams ground sample were dispersed in 50 ml of 10% acetic acid in methanol. The suspension was shaken and then allowed to stand for 4 h before it was filtered. The filtrate was evaporated to a quater of its original volume before concentrated ammonium hydroxide (30%) was added drop wise to precipitate the alkaloids. A pre-weighed filter paper was used to filter the precipitate and it was then washed with ammonium hydroxide solution (1%). The filter paper with alkaloid precipitates was dried at 60°C until a constant weight was obtained. The content of alkaloid was determined by the weight difference of the filter paper and expressed as g/100 g DW.

Statistical analysis

All analyses were carried out in triplicates and data expressed as means ± 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 various phytochemical content and antioxidant activity were done by using bivariate Pearson procedure.

RESULTS AND DISCUSSION

Antioxidant capacity

DPPH scavenging activity increased gradually with extract concentrations of Shandong PS, Menglembu PS and PRoot (Figure 1). Both Shandong PS and Menglembu PS showed significant DPPH radical scavenging activity with IC_{50} values of 3.2 and 3.1, respectively, while PRoot extract showed the highest DPPH radical scavenging activity with IC_{50} values of 1.9. The high DPPH radical scavenging activity exhibited by PRoot may be related with the high amount of different phytochemical constituents.

Nutritional composition

Peanut by-products contain high total dietary fiber with

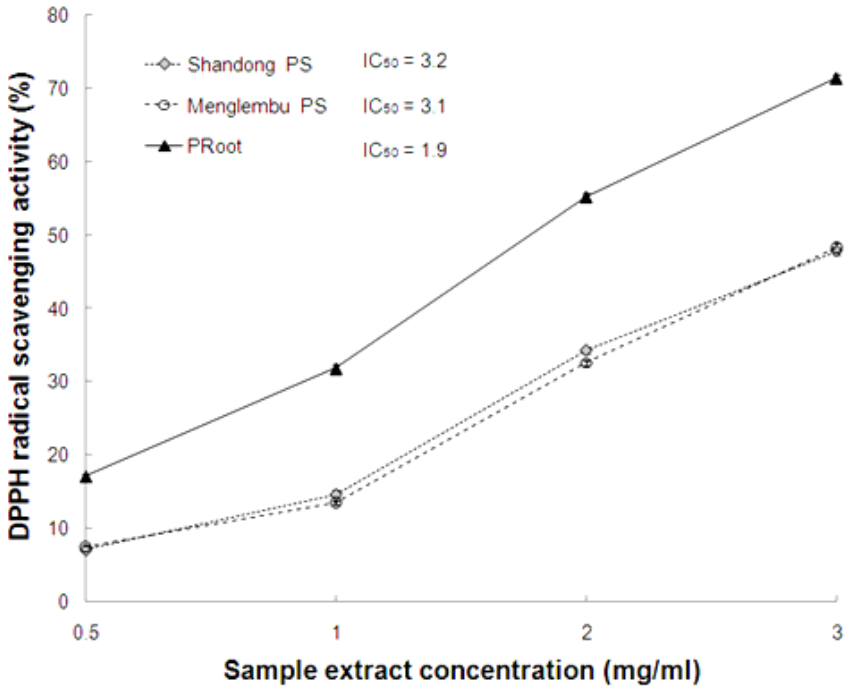

Figure 1. DPPH scavenging activity in extracts of Shandong PS (◇), Menglembu PS (○) and PRoot (▲) with respective IC_{50} values (n = 3)

Table 1. Nutritional and phytochemical content of peanut by-products.

Sample	Content[1]						
	Total dietary fiber[2]	Protein[2]	Ash[2]	Total phenolic[3]	Saponins[4]	Phytic acid[4]	Alkaloid[2]
Shandong PS	$58.8^{a} \pm 0.6$	$6.1^{a} \pm 0.5$	$21.7^{a} \pm 0.0$	$175^{a} \pm 3.9$	$10.8^{a} \pm 0.0$	$159^{a} \pm 11.8$	$4.5^{a} \pm 0.0$
Menglembu PS	$73.2^{b} \pm 0.6$	$5.8^{a} \pm 0.0$	$13.0^{b} \pm 0.2$	$255^{b} \pm 8.1$	$20.0^{b} \pm 0.0$	$108^{b} \pm 2.3$	$8.4^{b} \pm 0.0$
PRoot	$78.2^{c} \pm 0.4$	$6.1^{a} \pm 0.3$	$6.6^{c} \pm 0.2$	$431^{c} \pm 5.7$	$23.4^{c} \pm 0.0$	$149^{a} \pm 17.7$	$23.8^{c} \pm 0.0$

[1]Results were expressed as mean of triplicate measurements ± SD. Means with different superscript letters in a column are significantly different ($p<0.05$).[2]Values in g/100 g DW. [3]Values in mg tannic acid equivalent (TAE)/100 g DW. [4]Values in mg/100 g DW.

PRoot exhibiting the highest total dietary fibre content (78.2 g/100 g DW) (Table 1). Thus, it may exert prebiotics effects (Glenn, 2004) which warrant further research. However, these by-products showed a low protein content (from 5.8 to 6.1 g/100 g DW). Thus, protein supplements may be required if they are utilized as forage. Ash content is directly proportional to the amount of inorganic residues in a sample. Peanut shells and roots contain ash in the range of 6.6 to 21.7 g/100 g DW. Shandong PS would be a rich source of minerals as it showed the highest ash content.

Phytochemical content

PRoot showed the highest total phenolic, total saponins and alkaloids whereas Shandong PS had the lowest amount of these phytochemical. Phytic acid in peanut by-products exhibited no particular trend. Saponins can exert beneficial effects such as reducing cancer risks, lowering cholesterol levels, inhibiting dental caries and platelet aggregation and reducing the incidence of renal stones (Shi et al., 2004). However, dietary saponins from different plants were found to reduce growth (Bureau et al., 1998) and induce infertility (Qin and Xu, 1998). Phytic acid has been reported to show anti-cancer property besides its potential antioxidative effects (Graf, 1986). It can prevent kidney stone formation due to its mineral chelating potential and is capable to reduce cholesterol and triacylglycerol.

Excessive amounts of phytic acid in the diet may lead to the formation of insoluble complexes with multi-charged metals and results in mineral deficiencies (Nolan et al., 1987). Table 1 shows that total saponins and phytic

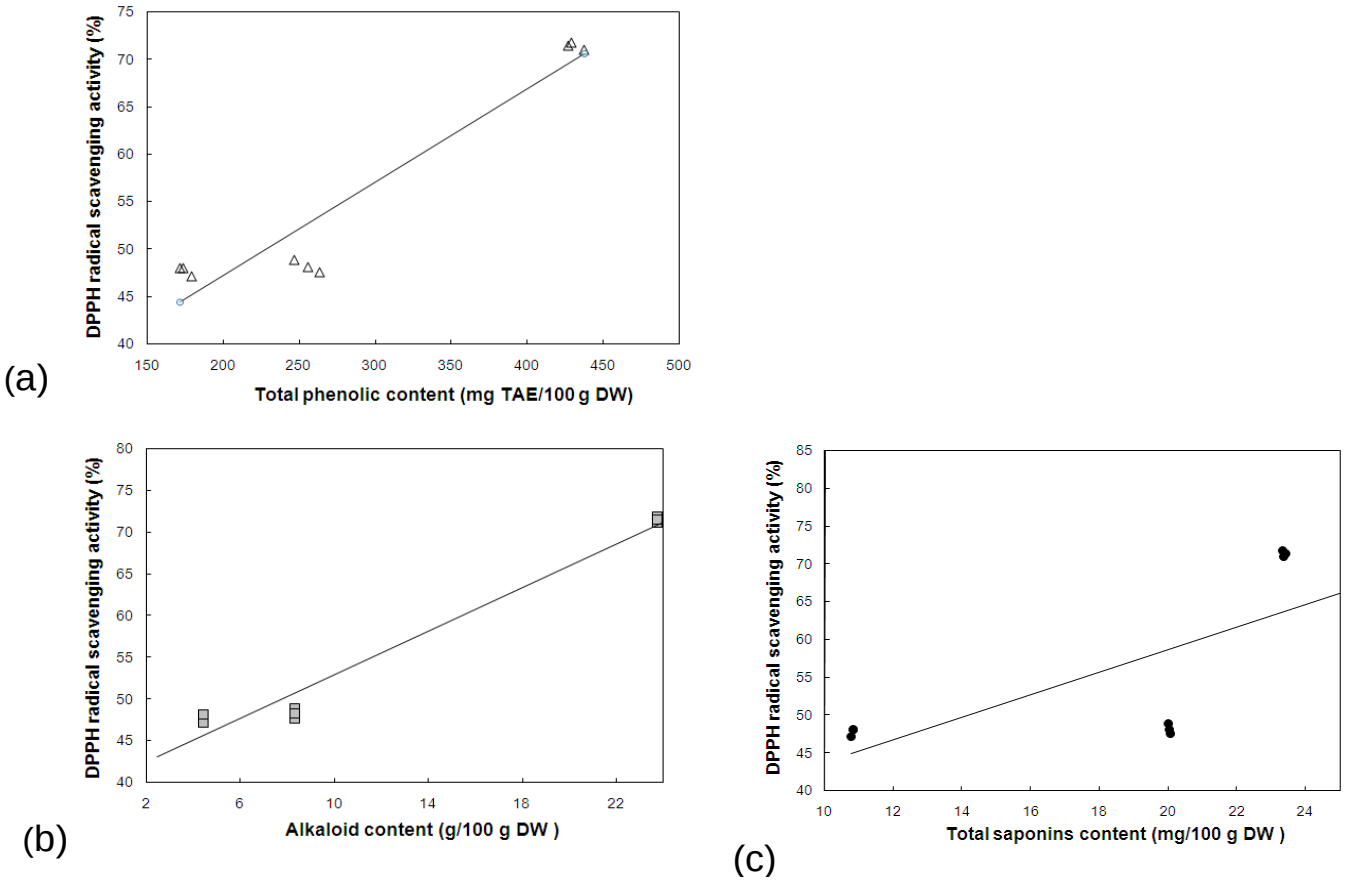

Figure 2. Correlations between phytochemical content and DPPH scavenging activity for (a) total phenolic (△); (b) alkaloid (□) and (c) total saponins (●) content (n=9, Pearson coefficient of 0.95, 0.98 and 0.72, respectively at p<0.01).

acid levels in peanut shells and roots were below the antinutritional limit of 1 mg/g and 500 mg/100 g, respectively (Francis et al., 2001). Thus these by-products can be assumed to be safe and unlikely to exert anti-nutritional effects on fishes and other organisms. Alkaloid content found in peanut shells and roots were within the range of some common foodstuffs (Adeniyi et al., 2009) and it is assumed that this amount is not prejudicial for animals. The alkaloid content in peanut roots was about two to three folds higher than in peanut shells which may be probably related with its distinct taste that protect the plant from herbivorous attack (Robert and Wink, 1998).

Relationship between phytochemical content and antioxidant capacity

Peanut roots had the highest phenolic, saponins and alkaloid contents. Positive correlations between these phytochemical contents and DPPH radical scavenging activity (from the extract whose concentration was 3 mg/ml) were found for peanut shells and peanut roots, with a Pearson correlation coefficient of 0.95, 0.97 and 0.72, respectively at p<0.01 (Figure 2). These finding are suggesting that:

i) Phenolic compounds that possess redox properties which lead to the antioxidative capacities (Siddhuraju and Becker, 2003) may be present in peanut shells and roots.
(ii) Specific alkaloid classes such as quinolone alkaloid with antioxidant activity (Chung and Woo, 2001) may be present in peanut shells and roots.
(iii) Saponins that act as chelators of transition metals (Cu^{2+} or Fe^{2+}) and results in diminished cellular sensitivity to oxidant damage (Amzal et al., 2008) may be present in peanut shells and roots.

In contrast, phytate content in peanut shells and roots was not correlated with DPPH radical scavenging activity (Pearson correlation coefficient of 0.28 at p<0.01, results not shown). Thus, phytic acid in peanut by products most probably would not exert antioxidative effect.

Conclusion

Shell and roots of peanut had a significant amount of total

dietary fiber, protein and ash content. Therefore, they can be utilized as feed or prebiotic compound. These peanut by-products also showed levels of total saponins and phytic acid below the antinutritional factor threshold. Therefore, feeding these peanut by-products would not produce detrimental effects on fishes or other living organisms. Peanut roots had the highest content of phytochemical and also showed the highest DPPH scavenging activity. Positive correlations were found between the phytochemical contents and the DPPH scavenging activity, implying that most phytochemical present in peanut roots may be utilized as antioxidants in nutraceutical sector or feed industries.

ACKNOWLEDGEMENTS

This study was supported by Universiti Tunku Abdul Rahman, Malaysia. Authors are thankful to the reviewers for their critical inputs on this manuscript.

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