

Nutraceutical evaluation and antioxidant potential of red kidney bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietenum*) seed coats

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Abstract

Legumes have become increasingly in demand due to the rich nutrient compositions and phytochemicals of pulses. However, the seed coats of some legume food products were removed prior consumption causing the food loss its nutritional value. The red kidney bean, RKB (*Phaseolus vulgaris*) and chickpea, CP (*Cicer arietenum*) which are the common beans among population were investigated in this study. Seed coats of these beans were analysed for the nutritional composition, phenolic compound and antioxidant properties. Carbohydrate was the major macronutrient in both seed coats. RKB and CP seed coats showed statistically significant composition of moisture, fat, protein and fibre. The caloric value of RKB seed coat (2.63 kcal/g) is higher than CP seed coat (2.29 kcal/g). Nevertheless, CP seed coat is a better source of fibre (27%) than RKB seed coat. Total phenolic content (TPC) of RKB seed coat was 12.14 mg GAE/g, which is much higher than in CP seed coat (0.25 mg GAE/g). Interestingly, the seed coat of RKB has strong antioxidant potency with DPPH assay ($IC_{50} = 105.18 \mu\text{g/ml}$) comparable to standard Trolox ($IC_{50} = 96.42 \mu\text{g/ml}$), which is much lower than the seed coat of CP ($IC_{50} = 606.12 \mu\text{g/ml}$). In addition, the antioxidant activity was highly correlated with TPC content of both seed coats. These properties make the seed coat of both beans are excellent candidates of potent nutraceutical.

Keywords: Antioxidant, Chickpea bean, Nutritional composition, Phenolic compounds, Red kidney bean

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Introduction

Wide range of countries populations have found an alternative for meat protein especially in the third

world countries. Due to growing awareness on nutritional-dependent sickness and costly expense for meat protein sources, consumers are improving their protein intake by choosing plant proteins specifically



legumes (Sasanam et al., 2011; Moses et al., 2012). Similar case happened for developing countries bearing protein malnutrition problem (Seidu et al., 2015). Beans are important staple and nutritious foods in the form of pods within the legume family that are consumed by humans (Polak et al., 2015). Beans are also referred as pulses like peas, beans, chickpeas and lentils which are said to be rich in sources of complex carbohydrates, proteins, vitamins and minerals. Lots of scientific data have shown that the consumers of pulse food are associated with lower risk of various chronic diseases such as diabetes, cardiovascular disease, cancer, obesity (Mudryj et al., 2012) and digestive disorder (Wang et al., 2010) which is attributable to the effect of naturally occurring antioxidant compounds and dietary fibers present.

Seeds are made up of embryo including cotyledons and the seed coat (maternal tissue). Commonly, the seed coat constitutes 9.9% of the bean entire dry matter whereas about 90.1% of the dry matter is represented by the embryo (Ribeiro et al., 2012). In addition, the colours of the seed coat play an important role in the determination of compounds present in it (Bajaj et al., 2015). Red kidney bean (*Phaseolus vulgaris*) is a common bean that contributes to 50% of the legumes consumed worldwide. Originated from Peru, kidney bean is the main source of protein in diet for several countries like Brazil, Uganda, America, East Africa and Asia (Amir et al., 2011; Ribeiro et al., 2012). Kidney beans which named upon its kidney-like shape are available in red, white and black colour but the mostly found is red kidney bean (Audu and Aremu, 2011). In contrast to dark colour of red kidney bean seed coat, a well-known pulse crop chickpea (*Cicer arietinum*) which is also called as 'Kacang Kuda' by the Malaysian, is the third globally important pulse crop production next to common beans and peas (Jukanti et al., 2012). There are two classes of chickpeas that are Desi and Kabuli. The Kabuli type is more common to consumers. It has thinner seed coat than the Desi type and the colour is much lighter like beige and almost white (Xu et al., 2014).

Upon food consumption, the seed coat of pulse crops are frequently being removed prior consumption or food product manufacturing. This happened due to the lack of knowledge and awareness among consumers on the nutritive values of outer coat of seed. Though several studies showed the antioxidant activity of some common beans, most of the information has been restricted to de-hulled seed (seed without its coat). To

the best of our knowledge, the nutritional content and antioxidant capacity of bean seed coats related to their nutraceutical value and health promoting effects are remain unexplored. Therefore, this study was aimed to investigate the proximate composition, caloric value, total phenolic and antioxidant potential of the seed coat of *Phaseolus vulgaris* (RKB) and *Cicer arietinum* (CP).

Material and Methods

Chemicals and reagents

All the chemicals used in this study were of analytical grade. Bovine serum albumin (BSA), Bradford reagent, Coomassie brilliant blue-G25, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, Folin-Ciocalteu reagent, gallic acid were purchased from Sigma-Aldrich (USA) and Merck (Germany).

Sample collection and preparation

Red kidney bean, RKB (*Phaseolus vulgaris*) and chickpea, CP (*Cicer arietinum*) were obtained from the BIG Supermarket, Sri Serdang, Selangor. The beans were soaked in distilled water for 4 hours to make the seed coats loose contact with the beans. Then, the beans were de-hulled (removal of seed coat from the seed) (Figure 1) by squeezing and the seed coats were sieved through 1 mm sieve. Then, the seed coats were air-dried for 48 hours in flat container for drying process. After drying, samples were grinded using a dry blender into powder form and stored at room temperature for further analysis.

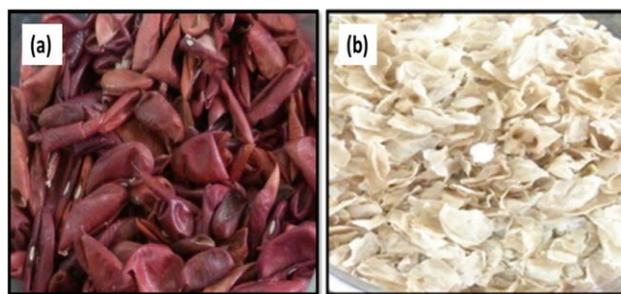


Fig.1. Red kidney bean (*Phaseolus vulgaris*) (a) and chickpea (*Cicer arietinum*) (b) seed coats.

Proximate composition

Moisture, ash, fat, fibre and carbohydrate contents were determined using method of Association of Official Analytical Chemists (AOAC) procedures (2005). Protein content was determined using

quantitative analysis of Bradford method (Bradford, 1976).

Determination of moisture content was according to AOAC method (2005) using the direct drying method. Homogenized sample (2 g) was dried in an air-oven set at 105°C for 3 hours until constant weight of the samples was obtained. The difference between initial weight and constant weight after drying was taken to be moisture lost and hence moisture content of sample. The samples were analysed in triplicates and the results are expressed as g/100 g samples.

Fat content of sample was determined using the solvent extraction method (Method No. 930.09) (AOAC, 2005). Ten gram of sample was extracted with petroleum ether on a Soxhlet apparatus at boiling point of 60°C - 80°C for 5 hours. The extracted fat in petroleum ether was collected and then fitted to rotary evaporator for separation of solvent from fat extract. The fat residue obtained was weighed. The samples were analysed in triplicates and the results are expressed as g/100 g samples.

Protein content of sample was determined according to the quantitative protein assay of Bradford method (Bradford, 1976). The isolation of protein from sample was initially prepared according to alkaline extraction method by Tounkara et al. (2013). The defatted flour of both seed coats were soaked in distilled water with ratio of 1:10 (w/v). The pH of solution was adjusted to 10 with 1 M NaOH at room temperature and the extract was then separated by centrifugation at 4,300 x g. The pH of extract was adjusted to 3.5 with 1 M HCl to allow precipitation and centrifuged at 4,300 x g for 20 minutes. The precipitate was washed twice with distilled water and re-suspended in the distilled water before being adjusted to pH 7.0 with 1 M NaOH prior to analysis. In the Bradford assay, a standard curve of BSA was prepared within the range of 0 to 2 mg/ml concentration. A 100 µl BSA was mixed with 5 ml Bradford reagent and incubated for 5 minutes. Absorbance value was measured at 595 nm and the calibration curve was plotted. The similar procedure was repeated using the protein extract from sample to determine the protein content based on the BSA calibration curve developed. The samples were analysed in triplicates.

Determination of ash content was according to Method No. 930.05 (AOAC, 2005). Ash content of sample was determined using the dry ashing method. 2 g sample was initially heated in the moisture extraction oven at 100°C for 3 hours. The heated

sample was then incinerated in a muffle furnace set at 550°C for 6 hours until greyish ash was obtained. Organic matter was burned off and the inorganic material remain was left to cool for overnight. The samples were then removed and were placed in desiccator to cool it to the room temperature. The samples were weighed as ash residue and analysed in triplicates.

Fibre was determined based on Method No. 930.10 (AOAC, 2005). Defatted sample (2 g) was added into 500 ml volume conical flask together with 200 ml of boiled 0.25 N H₂SO₄ to dissolve sample. The conical flask was then attached to reflux condenser and boiled for 30 minutes. The hydrolysed mixture was filtered by using No. 541 Whatman filter paper and rinsed by using boiled distilled water. The H₂SO₄ was substituted by 0.313 N of NaOH and the same condensing procedure was used. The residue was placed in oven for 3 hours at 105°C until constant weight was obtained. Then, the residue was placed in a muffle furnace set at 550°C overnight. The final weight was measured as fibre and analysed in triplicates.

The total carbohydrate content (%) was calculated by difference method (AOAC, 2005). The nitrogen free method was calculated as weight by difference between 100 and the summation of other proximate parameters as nitrogen free extract:

Percentage of carbohydrate,

$$\text{NFE (\%)} = 100 - (M + A + F1 + P + F2)$$

M = Moisture, A = Ash, F1 = Fat, P = Protein, F2 = Fibre

The caloric value was calculated using the Atwater system as suggested in previous study (Muzaffar et al., 2016). The weight of protein, fat and carbohydrate were multiplied by a factor of 4 kcal/g, 9 kcal/g and 4 kcal/g, respectively. The summation of the three values made up the caloric value was expressed as Calorie or kcal per 1 g of dry matter.

Caloric value = 4 kcal/g protein + 4 kcal/g carbohydrate + 9 kcal/g fat

Sample extraction for determination of antioxidant properties

Bean seed coats were extracted according to the method of Jun et al. (2012). Briefly, 10 g of sample was extracted with 200 ml of 40% acetone with continuous shaking on orbital shaker at 250 rpm for



12 hours at room temperature. Then, the extracted solution was centrifuged at 1000 x g for 15 minutes and filtered through a Whatman No. 4 filter paper. The filtrate was used for the analysis of total phenolic content. For DPPH scavenging activity, the filtrate was further evaporated by using rotary evaporator at temperature 50°C. The sample was then further dried in 60°C oven and was used to analyse total antioxidant activity.

Total phenolics content of sample extract was determined according to the method described by Dewanto et al. (2002). An aliquot (2 ml) from the filtrate was mixed with 1 ml Folin-Ciocalteu phenol (1:10 diluted with water) reagent and the mixture was allowed to stand for 5 minutes. Then, 3 ml sodium carbonate solution (10%, w/v) was added to the mixture. The end volume of the reaction mixture was made up to 10 ml with distilled water, vortexed and incubated in the dark for an hour. Then, the absorbance was recorded at 760 nm against a blank reagent. Gallic acid with concentration ranges from 0 to 400 µg/ml was used as standard. The analysis was performed in triplicates and the total phenolic content was expressed as mg of gallic acid equivalent/g of dry matter.

Free radical scavenging ability of the sample extract against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was evaluated as described by Williams et al. (1995). Briefly, serial dilution of the extracts and standard sample (100, 50, 25, 12.5 and 6.25 µg/ml) was prepared for standard curve. Sample (0.2 ml) was added to 2.8 ml of 60 µM ethanolic DPPH solution and mixed thoroughly. The mixture was then incubated at room temperature for 30 minutes in the dark. Absorbance of the reaction mixture was measured at 517 nm using UV spectrophotometer. Trolox was used as the standard and prepared based on the concentration of sample. The analysis was performed in triplicates. The results are expressed as percentage inhibition of radical scavenging activity as calculated using formula:

$$\text{Percentage of inhibition (\%)} = \frac{Ac - As}{Ac} \times 100$$

Ac = absorbance reading of control

As = absorbance reading of sample

Statistical analysis

Data are presented as mean ± standard deviation (S.D.) of three independent experiments. Statistical analyses were performed using statistical GraphPad Prism 7

software. Data were analysed by two-way analysis of variance (ANOVA) and the significance of the difference for each experimental test condition was assayed using t-test at a significance level of $p < 0.05$.

Results and Discussion

Nutritional composition of red kidney bean (RKB) and chickpea (CP) seed coats

The findings for proximate composition of RKB and CP seed coats are presented in Table 1. RKB seed coat was found to have significantly higher composition of fat (2.33 ± 0.21 g/100 g) and protein (16.77 ± 0.05 g/100 g) than in CP seed coat (0.83 ± 0.32 and 10.02 ± 0.16 g/100 g, respectively), while the ash content was not significantly different between both seed coats. Seed coat of CP was significantly higher in moisture content, contributed by the bulk tissue weight of coat layer. The moisture content obtained in CP seed coat was about two folds higher (13.33 ± 2.81 g/100 g) than obtained in RKB seed coat (6.67 ± 2.89 g/100 g). Among the compositions, carbohydrate accounted the most significant content in both RKB and CP seed coats with 44.90 ± 2.62 and 45.34 ± 2.60 g/100 g, respectively, which did not much differ between them. Thus, carbohydrate was shown as the major macronutrient in seed coats from both beans. However, the column factor of overall data show less or not significance between compositions.

Table. 1. Proximate composition of red kidney bean (RKB) and chickpea (CP) seed coats.

Beans seed coat	Proximate composition ^a				
	Moisture	Ash	Fat	Protein	Carbohydrate
Red kidney bean	6.67 ± 2.89	4.50 ± 0.50	2.33 ± 0.21	16.77 ± 0.05	44.90 ± 2.62
Chickpea	13.33 ± 2.81	3.33 ± 0.58	0.83 ± 0.32	10.02 ± 0.16	45.34 ± 2.60

^aProximate composition was expressed as g/100 g of dry matter

Each value represents the mean ± S.D. (standard deviation) of three determinations on dry weight basis.

Both RKB and CP seed coats were found to be higher in their moisture content than some lima bean seed coats ranged from 3.17% to 4.46% (Seidu et al., 2015). In another study of pulse food cowpea seeds, the chickpea remain the highest moisture content



among them (Antova et al., 2014). According to Moses et al. (2012), the moisture content of food is desirable at lower amount (not exceed 14%) to prevent any risk of mould growth and bacterial action which may develop hydrolytic rancidity. The ash content of the RKB and CP seed coats were 4.50 ± 0.50 and 3.33 ± 0.58 g/100 g, respectively, which correspond to several studies showing the low ash content of pulse food below 5% (Ghribi et al., 2015; Marathe et al., 2016). In addition, the available fat content was observed to be the lowest composition in both RKB (2.33 ± 0.21 g/100 g) and CP (0.83 ± 0.32 g/100 g) seed coats which is in agreement with previous studies demonstrating the lowest composition of fat in pulse food (Wani et al., 2014).

Pulse food is well-known for their good source of protein. Total protein content in both seed coats resulted 16.77 ± 0.05 g/100 g in RKB and 10.02 ± 0.16 g/100 g in CP, which accounted as the second highest of macronutrient available after carbohydrate. In fact, RKB seed coat occupied more than half amount of protein content of the whole seed. In a study of *Acacia totillis* seed, the carbohydrate content was found to be 45.30% which also the highest among other nutrient composition of the seed (Embaby and Rayan, 2016). In addition to other type of seed coat, a study of lima bean seed coats had found that carbohydrate content ranged from 47.11% to 48.62% were richer than the available carbohydrate in the seed coat of RKB and CP (Seidu et al., 2015).

The great level of carbohydrate found in both RKB and CP demonstrated the highest calorie contributor, followed by the total proteins and fat which affecting the determination of energy produced. Significant caloric value from the calculation of fat, protein and carbohydrate using the Atwater system resulted in 2.63 kcal/g and 2.29 kcal/g for RKB and CP seed coats, respectively (Table 2). The higher caloric value of RKB seed coat than CP seed coat was directly calculated from proximate composition of protein, carbohydrate and fat content as shown in Table 1. Thus, the more energy was provided by the RKB seed coat than the CP seed coat, as the determination of energy produced was highly affected by the total macronutrient contents (Ooi et al., 2012). Nevertheless, the findings indicated that both seed coats were rich in nutritional value which essential to be consumed as the whole seeds.

Nowadays, the interest in analysing the fibre content in food is increasing as it performs several beneficial effects such as lowering blood lipids level, prevention

of colon cancer and increasing the faecal transit time (Requena et al., 2016). In addition, the fibre intake may help in delaying the release of carbohydrate content which extending people feeling of fullness and improving the digestion as well (Tiwari et al., 2011). According to Table 2, fibre content was found to be significantly higher in CP seed coat (27.13 ± 0.29 g/100 g) than in the seed coat of RKB (24.83 ± 0.45 g/100 g). This result revealed positive correlation between fibre content and carbohydrate content as the fibre is a component type in carbohydrate. These two values are comparable with previous studies on the other types of seed coat, for instance, 32% to 33% of fibre content were found in lima bean seed coats and 33.72% in black gram seed coat (Girish et al., 2012; Seidu et al., 2015). In addition, the fibre content of both RKB and CP seed coats were higher than in other by-product such as the rice bran layer (10.97% to 13.51%) (Moongngarm et al., 2012).

Table. 2. Caloric value and fibre content of red kidney bean (RKB) and chickpea (CP) seed coats.

Beans seed coat	^a Caloric value (kcal/g)	^b Fibre (g/100 g)
Red kidney bean	2.63 ± 0.12	24.83 ± 0.45
Chickpea	2.29 ± 0.14	27.13 ± 0.29

^aTotal calorie was expressed as Calorie or kilocalories (kcal/g) of dry matter ^bFibre was expressed as g / 100 g of dry matter

Each value represents the mean \pm S.D. (standard deviation) of three determinations on dry weight basis.

Total phenolic content of RKB and CP seed coats

According to Figure 2, total phenolic content of RKB and CP seed coats were 12.14 mg GAE/g of dry matter and 0.25 mg GAE/g of dry matter, respectively. In a previous study by Fratianni et al. (2014), chickpea seeds accounted total polyphenols ranged from 147 to 183 μ g/g of dry matter which is in good agreement with the present study. In a review study by Singh and Basu (2012), the total phenolic compound in chickpea samples were slightly higher ranged from 0.92 to 1.68 mg GAE/g. A previous study reported that the phenolic compound of chickpea seed consisted of the nonflavonoid (hydroxybenzoic and hydroxycinnamic) and flavonoid (flavonols, flavanones and isoflavones) (Aguilera et al., 2011). In contrast to the light colour of seed coat of CP, the RKB seed coat is concentrated with phenolic compound that made up the dark and



highly pigmented bean seed coat such as anthocyanins, condensed tannins and flavonol glycosides. It was stated that the pulse with highly pigmented and dark varieties contain the highest polyphenolic content which aligned with the result obtained in this study (Dzomba et al., 2013).

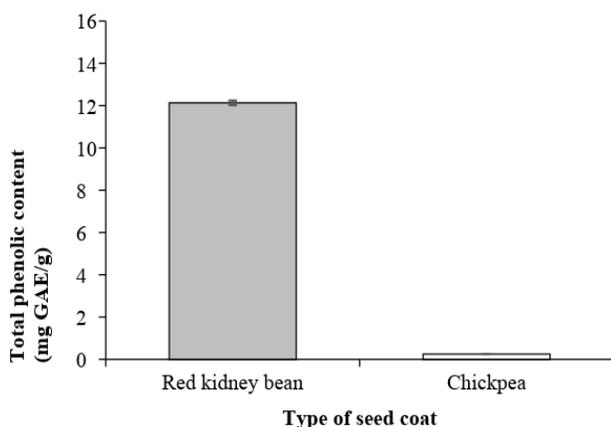


Fig. 2. The total phenolic content of red kidney bean (RKB) and chickpea (CP) seed coats. Total phenolic content was expressed as mg GAE/g. Error bars represent mean ± standard deviation (n=3).

Antioxidant capacity was measured based on the DPPH-radical-scavenging activity of both RKB and CP seed coats. The inhibition percentage performed by both seed coats extracts towards the free radical DPPH revealed the presence of antioxidant activity in both RKB and CP seed coats. Antioxidant activity present in the seed coats was determined and compared with the standard Trolox as shown in Figure 3, which the concentration at 50% inhibition was referred to IC_{50} value. Interestingly, RKB seed coat exhibited IC_{50} value of 105.18 $\mu\text{g/ml}$ which was significantly lower than the IC_{50} value exhibited by CP seed coat (606.12 $\mu\text{g/ml}$), thus indicating the less concentration of RKB seed coat was needed to achieve 50% inhibition of DPPH free radical than the CP seed coat. In addition, the IC_{50} value (105.18 $\mu\text{g/ml}$) of RKB seed coat showed comparable result with the standard Trolox (96.42 $\mu\text{g/ml}$), hence demonstrating the excellent free radical scavenging activity of RKB seed coat. In fact, antioxidant activity shown by both RKB and CP seed coats are directly proportional with the total phenolic content as shown in previous Figure 2, suggesting the greater the

antioxidant activity of RKB seed coat is contributed by the higher total phenolic content present.

The difference of IC_{50} value in both seed coats (Figure 3) could be explained by the darker colour (reddish-brown) of RKB seed coat as compared to the lighter colour of CP seed coat (yellowish-beige). This finding is also constant with a study on antioxidant activity in five coloured beans which reported that the highest activity was found in black bean, followed by red kidney bean, mung bean, soy bean and white bean. Therefore, it was suggested that the great content of anthocyanin, tannins and flavonoid (major dark pigment colour of bean seed coat) is highly responsible for the effectiveness of antioxidant activity against oxidative stress (Chutipanyaporn et al., 2014).

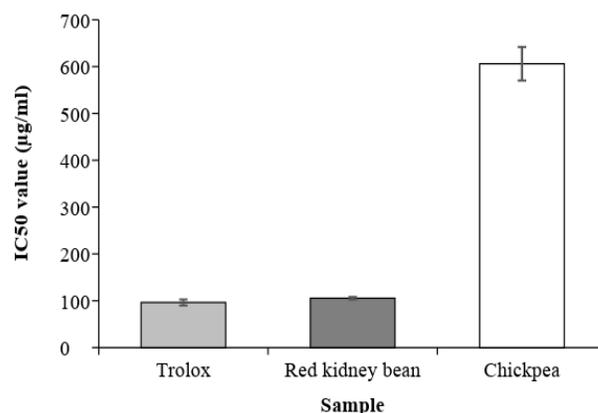


Fig. 3. The IC_{50} value of red kidney bean (RKB) and chickpea (CP) seed coats with comparison to the IC_{50} value of the standard Trolox. Error bars represent mean ± standard deviation (n=3).

Conclusion

RKB and CP seed coats containing considerable variability in the nutritional compositions (including caloric value and fibre content) and antioxidant properties were observed. RKB seed coat was suggestively appeared to be promising nutraceutical resource capable of yielding pulse seed coat with significant level of energy source and total phenolic content, demonstrating the most active antioxidant properties. Meanwhile, CP seed coat exhibited potent fibre source for functional food.

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Contribution of Authors

Zaidan UH: As the corresponding author and the supervisor; Leading the research project and research fund (PUTRA Grant-IPS9471300).

Karim NA: An undergraduate student who carried out the assigned project.

Ahmad S: The Co-author who gave permission to share the lab facilities for sample preparation.

Ghani SSA: The Co-author (research group member) who contributed ideas and discussion on project.

Halmi MIE: The Co-author (research group member) who contributed ideas and discussion on project.

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