Waste water of various boiled legumes as potential of radical scavenging agents

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Waste water of various boiled legumes as potential of radical scavenging agents

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Abstract. Peanut, soybean and chickpea important source of macronutrients and considered as important foodstuff in Malaysia. Boiled legumes are usually sold on by hawker on street. The water used for boiling the legumes is commonly thrown away without considering the radical scavenging potential of this so-called waste. Besides, there is lacking of awareness among the public about its nutritional benefits as part of sustainable food production aimed towards food security and nutrition. Furthermore, food antioxidants might play a significant role as physiological and dietary antioxidants which also could be a substitute for synthetic antioxidants and preservatives. Hence, this study aimed to evaluate the radical scavenging activity of the waste water from the three types of boiled legumes. The legumes were boiled in hot water and the waste water were collected. In this study, the waste water is converted into powder by means of freeze-drying technique. Radical scavenging activity was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, while its phenolic and flavonoid contents were determined using Folin-Ciocalteu assay and aluminum chloride colorimetric assay. The waste water of peanut showed the highest percentage of DPPH inhibition at 85.03±6.91%, followed by chickpea and soybean waste water at 79.89±3.69% and 69.10±6.19%, respectively. Furthermore, the waste water of peanut also showed the lowest IC_{50} value of 0.503±0.05 mg/mL, followed by soybean and chickpea of 0.554 ±0.01 mg/mL and 0.697±0.04 mg/mL. On a side note, peanut waste water extract showed the highest phenolic and flavonoid contents which are 1.877±0.36 mg GAE/g and 0.736±0.01 mg CE/g, respectively, whereas chickpea showed the lowest content of both which are 0.519±0.01 mg GAE/g and 0.227±0.01 mg CE/g, respectively. As conclusion, the waste water of boiled legumes is worth to be further investigated on their radical scavenging activity using other assays and could be potentially developed into functional foods.

1. Introduction

Nuts, legumes, seeds and pulses are all nutrient dense foods and have been a regular constituent of mankind's diet since pre-agricultural times [1]. The legume seeds or pulses, sometimes termed 'grain legumes', are second only to the cereals as a source of human food and provide the much-needed proteins to our predominantly vegetarian population. Not only legumes have been an important crop ever since man started domesticating plants, it has also been a major component of Mediterranean diet being also extremely important as part of our cultural heritage, like the Indian culture [2,3]. Boiled legumes are common foods that sold by hawkers on the street of Malaysia, and being favourite snacks among local community due to its best sources of plant-based proteins and also may help prevent risk factors for some chronic diseases. There are numerous studies regarding its radical scavenging activity and total phenolic content (TPC) which only focus on the seeds and shells. The water that had been

used to boil the legumes is discarded and thrown away after the boiling process, thus, being called as waste water. The potential of the waste water from various boiled legumes to become an alternative source of natural antioxidant is high due to issues regarding the use of synthetic antioxidant arise recently.

All the grain legumes are members of the family Fabaceae such as chickpea (*Cicer arietinum*), groundnut (*Arachis hypogaea*), as well as the soya bean (*Glycine max*). Peanut (*Arachis hypogaea*) is generally grown as a cash crop and used as a food source. The total production of peanut of country China is three times than other major producer nations which included Nigeria, India, United State, and Myanmar. It is suitable for human consumption and the seeds are easily handled such as can be eaten raw, boiled or roasted for directly consumption. Peanuts contain a great amount of monounsaturated fats which would reduce the risk of having heart attack compared to the intake of normal diet. The scientist's team of University of Florida had carried out an experiment and proven that a high concentration of phenols is found in the peanuts.

Soybean (*Glycine max*) is an annual crop and one of the most important plant in legumes family. Soybean provides a quarter of the worldwide edible oil, around 66% of the global protein concentrate for livestock feeding [4]. It is also one of the cash crops that in 2003, the export value of soybean in the United States was over 9.7 billion dollars [5]. According to the U.S. Food and Drug Administration, consuming around 25 grams of soy protein in a diet may lower the probability to having heart disease. Intake of soybeans might help to lower blood cholesterol and blood pressure, reduce menopause symptoms and decrease the risk of gaining certain types of cancer and osteoporosis.

Chickpea (*Cicer arietinum*) is one of the ancient and important crops that originated in South-eastern Turkey and now has been grown in more than 30 countries in different region of Asia, Africa, Europe, United States and Australia [6]. Chickpea has high demand in making hummus and act as supplements for famine-stricken region and beneficial to prevent and management metabolic syndrome [7]. It is a popular crop due to its less consumption of water compared to other popular crop. This characteristic has made chickpea become popular crops in Ethiopia and recognized as Africa's largest chickpea producer [8].

2. Materials and Method

2.1. Chemicals and reagents

Ascorbic acid, Folin-Ciocalteu, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), methanol, catechin, aluminum chloride ($AlCl_3$). All chemicals and reagents are from Sigma-Aldrich.

2.2. Sample collection and preparation

The peanut was purchased at a grocery in Machang, Kelantan, while the soybean and chickpea were purchased at a morning market in Jeli town. The raw peanuts were cleaned with tap water, while the spoiled and perished leguminous fruit were removed. 500 g leguminous fruit were boiled with 1.5 L water for 60 minutes, and removed from the hot water. The water that used to boil the sample, which later called as waste water was collected and kept in a -80 ^{o}C freezer for overnight, before further process with a freeze dryer. The extraction process was carried out with the dried samples were dissolved in methanol in different concentrations; 1.00, 0.80, 0.60, 0.40, 0.20 mg/mL.

2.3. Free radical scavenging capacity

The free radical scavenging assay of the waste water from samples were adopted from Dhianawaty & Panigoro, and Win *et al.* [9,10] with slightly modification. Free radical activity of the waste water from samples were compared with standard compound. A 0.004% of DPPH stock solution was prepared by dissolving the 0.004 g of the DPPH in 100 mL of methanol.

Each extract (1 mL) was mixed with 1 mL of 1.0 x 10^{-3} M concentration of DPPH solutions. Then, the mixture was mixed thoroughly using a vortex and incubated for 30 mins, prior to the absorbance

reading. The absorbance of the samples, positive control and a blank were recorded by a spectrophotometer (UV-Vis 2450 Shimadzu, Japan) at 517 nm. The positive control used in this study was ascorbic, ranging between of 0.01-0.40 mg/mL, while blank was 1 mL sample plus 1 mL methanol, and negative control was 1 mL DPPH plus methanol. The percentage of DPPH inhibition was calculated using the equation below:

$$\text{DPPH radical scavenging capacity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorban of Sample}}{\text{Absorbance of Control}} \text{ x } 100\%$$

Absorbance of control is the absorbance of the DPPH radical in methanol
Absorbance of sample is the absorbance of mixture DPPH radical solution and sample extract.

2.4. Total phenolic contents (TPC)
The total phenolic content of the extracts was determined using the Folin Ciocalteau assay with slight modifications according to the method by Singleton & Rossi [11]. Gallic acid was used as a standard and had the calibration curve plotted. A 2.0 mL of 10 folded diluted Folin-Ciocalteu phenol reagents were mixed with each 0.4 mL methanolic sample in a test tube. The mixture was left at room temperature for 10 mins. After 10 minutes, the mixture was added with 1.6 mL of 7.5% (w/v) sodium carbonate solution and mixed gently. The mixture was then incubated for 30 mins, followed by the absorbance reading at 725 nm on a spectrophotometer. The total phenolic content was showed in milligrams of gallic acid per gram of dry sample (mg GAE/g).

2.5. Total flavonoid contents (TFC)
The determination of the flavonoid equivalent of the extracts was performed according to the method by Norra [12]. Catechin was used as a standard and had its calibration curve plotted. In a 10 mL test tube, a sample of 0.3 mL and 0.15 mL of 0.5M $NaNO_2$ were mixed. After 4 minutes, 0.15 mL of 10% $AlCl_3$ was added. After 1 minute, 0.1M NaOH was added into the mixture. After 5 minutes incubation at room temperature, absorbance reading was measured against the blank solution at 506 nm. The total flavonoid content was showed in milligrams of catechin per gram of dry sample (mg CE/g).

2.6. Statistical analysis
All experimental groups in all sections above were conducted in triplicates. The results are presented as means±S.D. The IC_{50} values were obtained by linear regression (MS Excel) and showed a good coefficient of determination ($R^2 \geq 0.90$). Data was analysed using SPSS version 20.0. Statistical significance between groups was calculated by analyses of variance (ANOVA), followed by Tukey multiple comparisons test. P-values less than 0.05 ($p<0.05$) was considered as statistical different.

3. Results and Discussion

DPPH free radical scavenging assay was used to investigate the free radical scavenging capacities of the waste water from three types of boiled legumes and were compared with the standard ascorbic acid. All samples had shown positive results of inhibition activity. Figure 1 and 2 show the radical scavenging activity (%) of the ascorbic acid (positive control) and the waste water of the boiled legumes measured at absorbance of 517 nm. Table 1 shows the percentage of DPPH inhibition activity with ascorbic acid showed the highest activity at 97.34±0.34%, followed by peanut, chickpea and soybean waste water at 85.03±6.91%, 79.89±3.69% and 69.10±6.19%, respectively. The IC_{50} values of the waste water obtained from the equation of linear regression, in ascending order are peanut waste water at 0.503±0.05 mg/mL, followed by soybean and chickpea waste water at 0.554 ±0.01 mg/mL and 0.697±0.04 mg/mL, respectively. The lower IC_{50} value indicates the higher inhibition, thus, the higher scavenging activity.

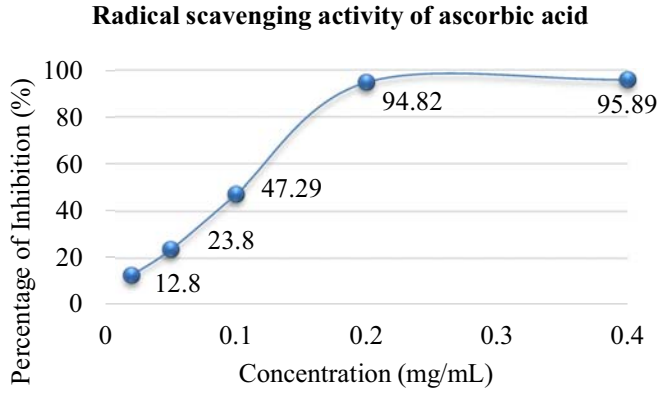

Figure 1. Radical scavenging activity of ascorbic acid obtained from equation of linear regression at 517 nm.

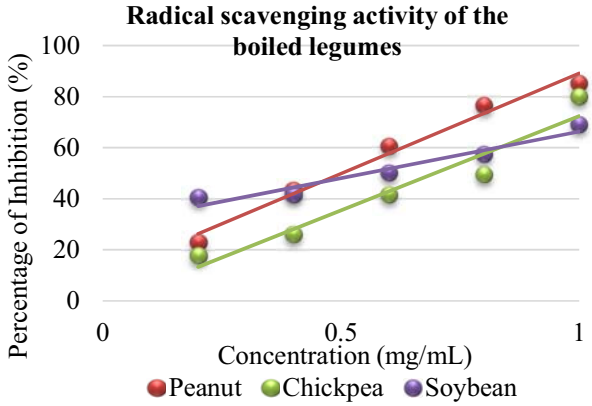

Figure 2. Radical scavenging activity of the waste water from various boiled legumes obtained from equation of linear regression at 517 nm.

Table 1. IC_{50} values (mg/mL) of the waste water of various legumes and positive control, ascorbic acid obtained from the equation of linear regressions at 517 nm.

Sample	Percentage of Inhibition (%)	IC_{50} (mg/mL)
Ascorbic Acid	97.34±0.34	0.11±0.01
Peanut	85.03±6.91	0.50±0.05
Chickpea	79.89±3.69	0.69±0.04
Soybean	69.10±6.19	0.55 ±0.01

*Values were expressed as mean ± standard deviation (n=3). The IC_{50} values were obtained by linear regression and showed a very good coefficient of determination ($R^2 \geq 0.90$). $p<0.05$ were used as the significant level when compared to the positive control ascorbic acid.

The use of DPPH provides an easy and rapid way to evaluate antioxidant activity [13]. Any substance that can donate a hydrogen radical (antioxidant) to the solution of DPPH can reduce the stable free radical and change the colour of its solution from violet to pale yellow [11]. In the spectroscopic method, the result of antioxidant efficiency is expressed as IC_{50} determined as the concentration of substrate that causes 50% loss in absorbance (DPPH activity) [14]. High antioxidant

activity showed by the peanut waste water could be contributed by the polyphenols, as reported by Ozer [15]. The highest IC_{50} value which indicates the lowest activity exhibited by the chickpea in this study, is in agreement to the study by Sushama *et al.* [16] who was also reported that the chickpea, both the cream and green ones showed low antioxidant activity in the DPPH, $ABTS^{\bullet+}$ and FRAP assays.

Furthermore, it can be seen that the percentage of inhibition of the waste water are varies which could be influenced by several factors such as temperature, light, air, physical and chemical properties of the substrates, and the presence of oxidation catalysts or itiators. The use of polar solvent (water) as the extractant in this study could also contribute to the antioxidant activity as stated by Xu and Chang [17].

3.1. Total phenolic content (TPC)

The total phenolic content of the waste water was determined using the gallic acid as a standard and had the calibration curve plotted using the absorbance values obtained with its solutions ranging between 0.00 mg/mL and 0.40 mg/mL (Figure 3).

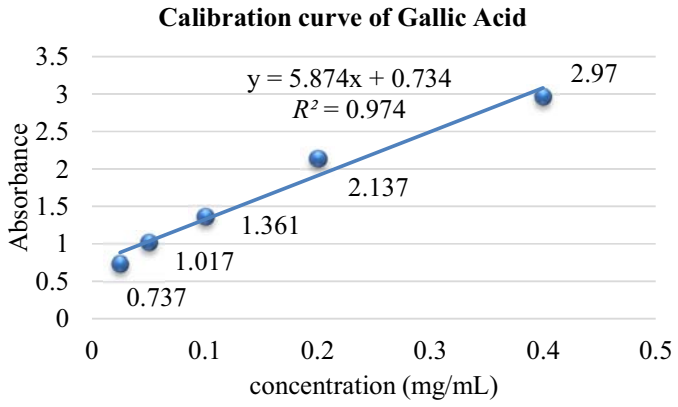

Figure 3. The calibration curve of gallic acid used as a standard reference in the total phenolic content assay.

The waste water of boiled legumes were tested for their total phenolic content using the same procedure used for the standard as shown in Figure 4. The results were expressed in milligrams of gallic acid per milliliter of extract (mg GAE/g), with the TPC of peanut waste water was the highest at 1.877±0.04 mg GAE/g, followed by soybean waste water at 1.635±0.05 mg GAE/g, and chickpea waste water at 0.519±0.01 mg GAE/g. Data also showed significant difference (p<0.05) between all sample tested.

Phenolic compounds could react with the molybdenum-containing Folin-Ciocalteu reagent and might be induced by an electron transfer during the TPC assay. With the electron transfer, the deep yellow colour was converted to a blue colour, which can be measured spectroscopically. In this study, the waste water from peanut contains the highest phenolics at 1.877±0.04 mg GAE/g (equivalent to 187.7 mg GAE/100 g) (Figure 4). This is contrast to a report by Ozer [15], who reported higher phenolic contents ranging between 326 to 552 mg GAE/100 g for the peanut. However, the different value could be affected by the different geographical locations of the legumes, the extraction solvent, or the time used in their studies [15]. The good correlation between phenolic compositions and antioxidant activities of the peanut extracts is supported by studies by Xu and Chang [16,17].

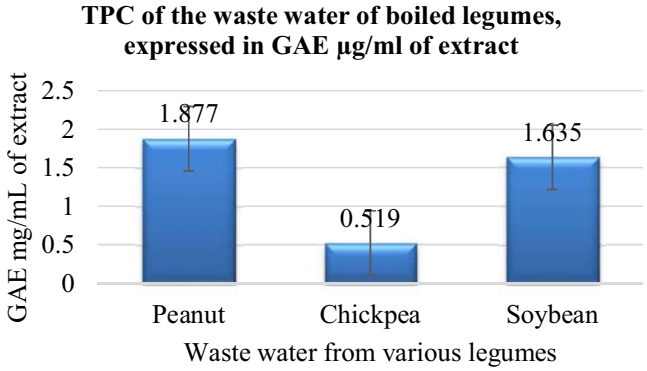

Figure 4. Total phenolic content (TPC) of the waste water of boiled legumes expressed in GAE µg/ml of extract. Values were expressed as mean±standard deviation (n=3), calculated based on the equation of the gallic acid callibration curve.

3.2. Total flavonoid content (TFC)

Moreover, the total flavonoid content of the waste water was determined using the catechin as a standard and had the calibration curve plotted using the absorbance values obtained with its solutions ranging between 0.10 mg/mL to 1.60 mg/mL (Figure 5).

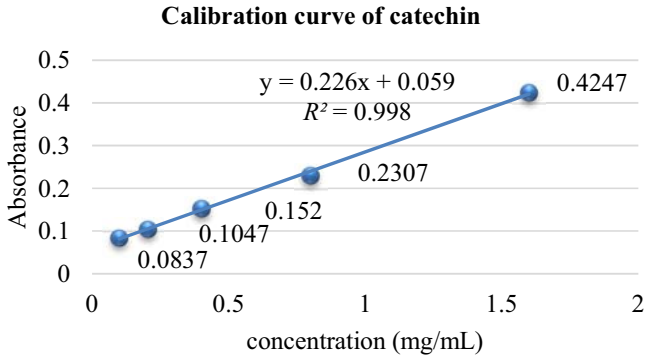

Figure 5. The calibration curve of catechin used as a standard reference in the total flavonoid content assay.

The waste water of boiled legumes were tested for their total flavonoid content using the same procedure used for the standard as shown in Figure 6. The results were expressed in milligrams of catechin per milliliter of extract (mg CE/g), with the highest TFC showed by peanut waste water at 0.736±0.01 mg CE/g, followed by soybean and chickpea waste water at 0.350±0.02 mg CE/g and 0.227±0.01 mg CE/g, respectively. Data also showed significant difference (p<0.05) between all sample tested.

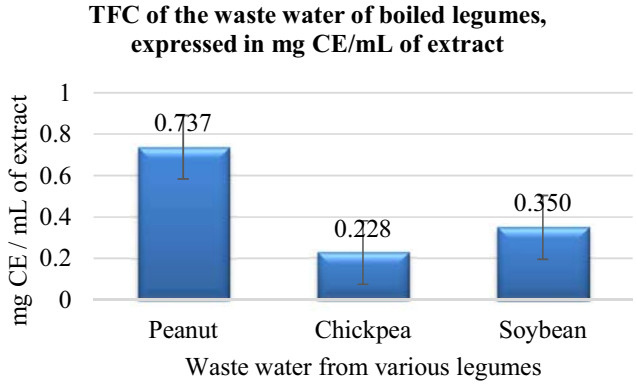

Figure 6. Total flavonoid content (TFC) of the waste water of boiled legumes expressed in mg CE/g of extract. Values were expressed as mean ± standard deviation (n=3), calculated based on the equation of the quercetin calibration.

Various absorption abilities of each type of flavonoid compound as the number and position of the sugar moiety could caused different influences on the activity of the flavonoids [15,17]. Preparation of the flavonoid-contained food for consumption can also lead to some loss in flavonoid content. Although flavonoids are relatively stable compounds, resistant to heat, oxygen and moderate degrees of acidity, kitchen preparation will cause some flavonoid losses [15].

It is well known that the antioxidant activity of plant materials is well correlated with the phenolic content [18]. In this study, the highest DPPH inhibition effect exhibited by the peanut waste water showed good correlation with its phenolic and flavonoid contents. The findings are in agreement with Segura *et al.* [21] and Bodoira & Maestri [22] who reported that bioactive constituents of legumes, such as phenolic acids, flavonoids, tannins, phenolic lignans, and stilbene derivatives play important roles in which oxidative stress reactions are involved, which explains the high free radical inhibition in the antioxidant activity. Other study also showed reduction of lipid peroxidation or oxidative DNA damage with nut extracts in *in vitro* studies and the beneficial effects of nut intake on lipid oxidation, antioxidant enzyme activity, and formation of cholesterol oxidation products in both acute and chronic experimental animal studies [23].

The use of water as the extractant in this study has extracted most of the polar compounds from the legumes as stated by Ozer [12], thus explained the activity. In addition differences identified among the tests ran by different samples may be attributed to differences in extraction and hydrolysis times and temperatures, or the fore mentioned preharvest factors such as climate, geography, or agronomic practices [24]. In addition, the biologically-active compounds of interest found in leguminous seeds come from many chemical classes including the phenolic compounds as well as their derivatives [25].

4. Conclusion

The waste water of peanut showed the highest percentage of DPPH inhibition with the lowest IC_{50} value, as well as the highest phenolic and flavonoid contents The waste water of boiled legumes are worth to be further investigated on their radical scavenging activity using other assays and could be potentially developed into functional foods.

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