

Research Article

Antioxidant and antigenotoxic activities of phenolic-enriched extracts from legume seed coats

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Abstract

Seed coats of some legumes (mung bean, peanut and soybean) are a low economic value by-product of the legume product manufacturing industry in Thailand. The objective of this work was to determine total phenolic and flavonoid contents, antioxidant activity and antigenotoxic activity of legume seed coats (black bean, mung bean, peanut, red kidney bean and soybean) for developing value-added products that have health-benefits. To evaluate antioxidant activities, seed coat extracts were tested using DPPH and FRAP assays. The protective effect of the seed coats on H₂O₂ induced DNA damage in lymphocytes was analyzed by Comet assay. The results found that the seed coats of peanut and red kidney bean displayed high levels of total phenolics and flavonoids. All tested seed coats except soybean showed a high level of DPPH scavenging effect (>80%). High FRAP values were found in the seed coats of peanut and red kidney bean. Moreover, all processed seed coats except mung bean displayed strong inhibition (>60%) on DNA damage induced by H₂O₂. In conclusion, all the by-products could provide an inexpensive source of natural antioxidants and antigenotoxic compounds.

Keywords: waste recovery, value addition, DPPH, FRAP, comet assay, human lymphocytes, Thailand

Introduction

Legumes play an important role in the traditional diets of many regions throughout the world. Three different parts are recognized in the legume seeds: cotyledon, seed coats and embryonic axis, which represent, on average, 89%, 10% and 1%, respectively, of the total seed weight. The seed coat has the highest concentration of phenolic compounds [1]. Polyphenolic compounds from dry beans may possibly act as antioxidants and antigenotoxins.

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Materials and Methods

Sample preparation

Seed coats from black bean, red kidney bean, mung bean, peanut and soybean were divided into 2 groups. The first group was used as raw samples, whereas the second group was processed by autoclaving at 121°C for 20 min. For extraction, two grams of the seed coat powder was extracted with 40 ml of 70% acetone for 24 h, followed by filtration. The filtered extracts were concentrated in a vacuum evaporator at 40°C. The concentrated extracts were stored in a freezer at -20°C. For each experiment, the extracts were re-suspended in 10% dimethyl sulfoxide to give the appropriate concentrations for each assay.

Determination of total phenolic content

The total phenolic content of the extracts was determined according to the method described by Amarowicz *et al.* [2]. Briefly, 10 µl of each extract was transferred into a 96-well microplate containing 160 µl of distilled water. After mixing the contents, 10 µl of Folin–Ciocalteu reagent and 20 µl of a saturated sodium carbonate solution were added. The solution was mixed well and the absorbance was recorded at 750 nm with microplate reader (Sunrise, Tecan Co., Austria) after 30 min incubation. The total phenolic content was calculated from a calibration curve of gallic acid solutions (ranging from 25 to 800 mg/l), and were expressed as milligram of gallic acid equivalent (GAE) per gram of the extract. All measurements were done in triplicate.

Determination of total flavonoid content

Flavonoid content in the extracts was determined by a colorimetric method described by Jia *et al.* [3], with some modifications. The 250 µl of extract was mixed with 1.25 ml of distilled water and 75 µl of 5% NaNO₂ solution in a test tube. After 5 min of incubation, 150 µl of 10% AlCl₃.H₂O solution was added and incubated for 6 min. Then, 500 µl of 1 M NaOH and 275 µl of distilled water were added. The solution was mixed well and the absorbance was read at 510 nm. Catechin was used to calculate the standard curve (0.25 –2.5 mM) and the results were expressed as milligram of catechin equivalents (CE) per gram of the extract.

Scavenging of 2, 2'-Diphenyl-1-picrylhydrazyl Radical (DPPH Assay)

The extract was allowed to react with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in order to evaluate the free radical scavenging activity using the procedure described by Fukumoto and Mazza [4]. An aliquot of 22 µl of the extract or blank reagent (10% DMSO) or standard Trolox (0.04-1.28 mM in 80% methanol) was added to 200 µl of DPPH in 80% methanol (150 µM) in a 96-well microplate. After incubation at 37°C for 30 min, the absorbance was read in a microplate reader using a 520 nm filter. DPPH scavenging activity (%) = 100 × [1 - (A_E/ A_D)], where A_E is the absorbance of the solution containing DPPH and extract, and A_D is the absorbance of the DPPH solution with nothing added.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity was measured by its ability to reduce the Fe³⁺-TPTZ complex by forming Fe²⁺-TPTZ. Each of 20 µl of the extract or standard (ferrous sulfate) or blank reagent (10% DMSO) was added to each well of a 96-well microplate. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyls-triazine (TPTZ)

solution and 20 mM FeCl₃.6H₂O in a 10:1:1 ratio prior to use and heated to 37°C in a water bath according to the procedure described by Griffin and Bhagooli [5]. The 150 µl of FRAP reagent was added to each well. The absorbance at 600 nm was performed after 8 min of incubation using a microplate reader.

Single cell gel electrophoresis (Comet assay)

To evaluate the protective effect of the extracts on H₂O₂ induced DNA damage, the extracts were introduced into microcentrifuge tubes; RPMI 1640 medium and lymphocyte suspension were added and incubated at 37°C for 25 min. After incubation, the suspension was treated with 1 mM H₂O₂ for 5 min on ice. Then the lymphocytes were used for viability test and Comet assay [6]. The lymphocyte pellet was re-suspended in 100 µl of 0.8% low melting-point agarose. Then 30 µl of this cellular suspension was spread onto slides that had previously been coated with 1.0% normal melting point agarose. The slides were immersed in lysing solution for at least 1 h. Following this the slides were immersed in alkaline electrophoresis buffer (0.3 M NaOH and 1 mM Na₂EDTA, pH > 13) at 4°C for unwinding (40 min) and then electrophoresed (26 V/ 300 mA, 20 min). Silver staining was done according to the method of Garcia *et al.* [7]. The evaluation of DNA damage was carried out by the visual score under a microscope [8].

Results and Discussion

Total phenolic and total flavonoid contents

The seed coats of peanut and red kidney bean displayed high content of total phenolics (>300mg GAE/g extract) and total flavonoids (> 200 mg CE/g extract) (Figure 1). Heat treatment could affect the levels of total phenolic and total flavonoid contents.

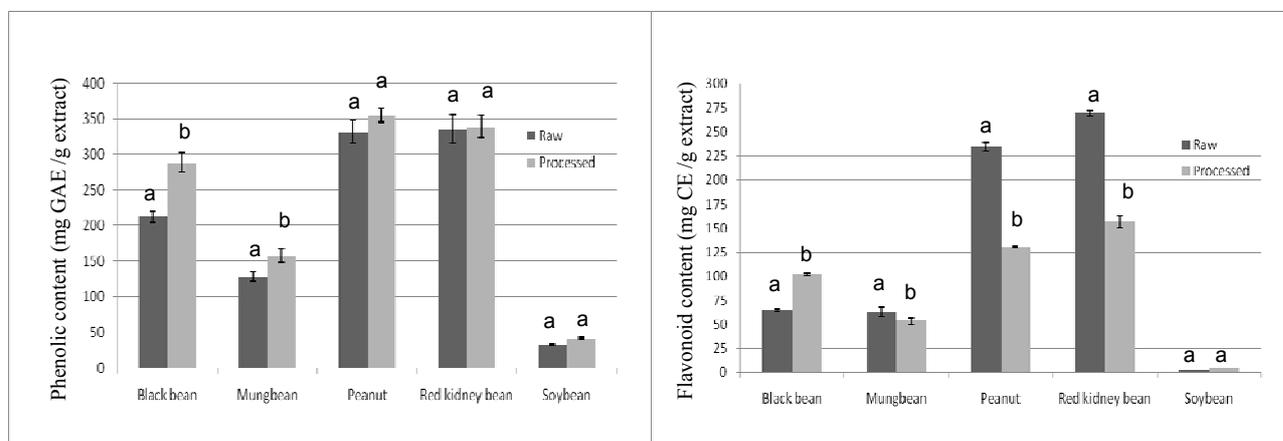


Figure 1. Effect of heat treatment on total phenolic and flavonoid contents of seed coat extracts.

Antioxidant activities

Seed coat extracts displayed a high level of DPPH scavenging effect (>80%) except soybean (<20%) (Figure 2). The seed coat of peanut and red kidney bean had high FRAP value (Figure 3). After heat treatment, ferric reducing power of seed coat of black bean was increased, but that of the other seed coat was decreased.

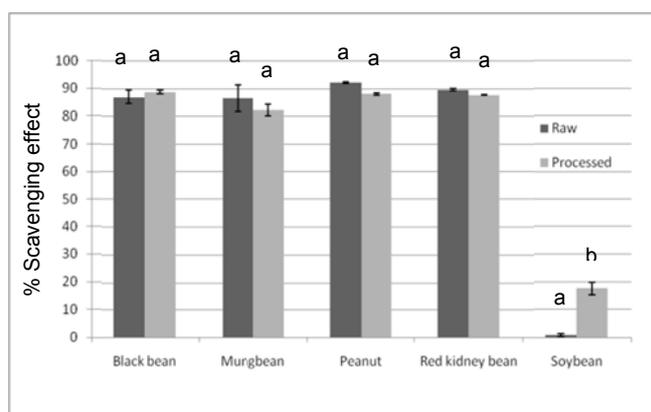


Figure 2. % of DPPH scavenging activity.

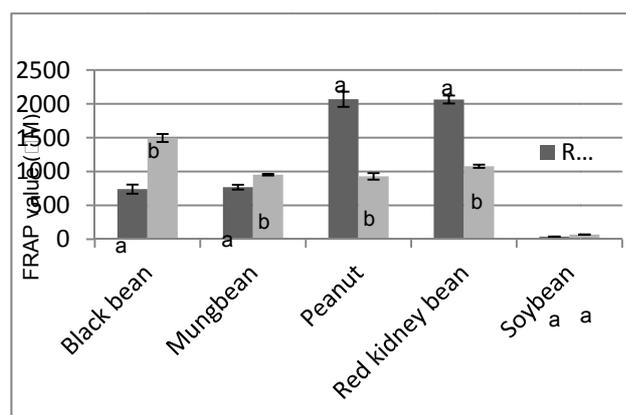


Figure 3. FRAP value (ferric reducing power).

Single cell gel electrophoresis (Comet assay)

The inhibitory effect of each seed coat extract on the DNA damage of H_2O_2 is shown in Table 1. At the concentration of $1 \mu\text{g/ml}$, the maximum inhibitory effect of DNA damage was observed for exposure to the extracts of processed seed coat (43.0- 63.3%), whereas the inhibitory effect was weaker when exposed to the extracts of raw seed coat (24.9-53.7%).

Table 1. Effects of the seed coat extracts on DNA damage induced by H_2O_2 in human lymphocytes.

Sample	Conc. ($\mu\text{g/ml}$)	Seed coats extracts			
		Raw		Processed	
		AU	%Inhibition	AU	%Inhibition
Water	-	14 ± 2.1		14 ± 2.1	
H_2O_2 (20 μM)	-	287 ± 37.5		251 ± 35.4	
Black bean	0.04	149 ± 25.5	48.1	$127 \pm 17.0^*$	49.4
	0.2	165 ± 21.2	42.5	$119 \pm 8.5^*$	52.6
	1	$133 \pm 26.2^*$	53.7	$92 \pm 15.6^*$	63.3
Mung bean	0.04	206 ± 18.4	28.2	198 ± 38.2	21.1
	0.2	201 ± 21.2	30.0	180 ± 19.8	28.3
	1	196 ± 14.8	31.7	$117 \pm 21.2^*$	53.4
Peanut	0.04	208 ± 30.4	27.5	139 ± 14.1	57.0
	0.2	218 ± 11.3	24.0	145 ± 15.6	55.1
	1	174 ± 18.4	39.4	$118 \pm 29.7^*$	63.5
Red kidney bean	0.04	174 ± 26.9	39.4	$166 \pm 18.4^*$	33.9
	0.2	$150 \pm 18.4^*$	47.7	$123 \pm 22.6^*$	51.0
	1	$139 \pm 14.1^*$	51.6	$96 \pm 15.6^{**}$	61.8
Soybean	0.04	204 ± 29.0	28.9	177 ± 19.1	45.2
	0.2	162 ± 36.8	43.6	$118 \pm 11.3^*$	63.5
	1	182 ± 33.2	36.6	$124 \pm 15.6^*$	61.6

Arbitrary unit score (AU) showed mean \pm S.D. of two independent experiments.

* $p < 0.05$ refers to differences between H_2O_2 -treated lymphocytes preincubated with or without sample extracts.

Discussion

The seed coat which contained high phenolic content was shown to have higher antioxidant activities than the seed coat that contained low phenolic content. Phenolic compounds have been reported to have higher antioxidant activities, as compared to the common antioxidants, vitamin C and vitamin E [9]. After heat treatment, the decrease of antioxidant activities of the extracts was correlated with the decrease in flavonoid content rather than the increase of total phenolic content. The increase of total phenolic content might be due to the increase of non-flavonoid compounds from the breakdown of cellular constituents. In addition, the result suggested a positive correlation between high level of phenolic contents and antigenotoxic effect. Several works have reported that phenolics exhibited antigenotoxic effect by contributing to scavenging of reactive oxygen species. Ruch *et al.* [10] proposed that H₂O₂ might be scavenged by phenolics, which could donate electrons to H₂O₂ and neutralize H₂O₂ to water. Moreover, several reports found some phenolic compounds in legumes such as anthocyanins in black beans [11], or resveratrol [12] and procyanidins in peanut. These phenolic compounds might be the compounds responsible for inhibition of DNA damage in human lymphocytes induced by H₂O₂ in this study.

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