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Evaluation of the Antioxidant Activity of Legumes

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ABSTRACT

The anti-lipid peroxidation activity, free radical scavenger activity and anti-superoxide formation of hot water extracts of legumes (HWEL), such as mung bean (*Phaseolus radiatus* L.), adzuki bean (*Phaseolus aureus* Roxb.), black bean (*Glycine max* (L.) Merr.) and rice bean (*Phaseolus calcaratus* Roxb.), were evaluated to test antioxidant activity. The results showed that all HWEL exhibited remarkable inhibition of $FeCl_3$-ascorbic acid induced lipid peroxidation of mouse liver homogenate . All extracts showed anti-lipid preoccupation activities. Both mung bean and adzuki bean extracts demonstrated the strongest anti-lipid peroxidation activity and the highest superoxide anion scavenging activity. Therefore, a component of the diet, HWEL, is a potential antioxidant.

INTRODUCTION

Lipid peroxidation not only lowers the nutritive value of food and deteriorates the taste and flavor, but may contribute to aging, coronary heart disease, diabetes mellitus, stroke, rheumatic disease, various liver disorders and carcinogenesis. Leguminous seeds such as mung bean (*Phaseolus radiatus* L.), adzuki bean (*Phaseolus aureus* Roxb.), black bean (*Glycine max* (L.) Merr.) and rice bean (*Phaseolus calcaratus* Roxb.), are widely distributed in tropical and subtropical beaches and native to southern Taiwan (Tainan, Kaohsiung, Pintung). They have been regarded as folk medicines and foods or beverages in the daily diet, and widely used in the treatment of antidote, edema, diuretic, antifebrile, carminative, etc. Legumes can be utilized as valuable ingredients of various products. Generally, beverages are prepared from hot water extracts of legumes (HWEL). However, it remains unclear if HWEL possess antioxidant activity. Therefore, this study was undertaken to evaluate the antioxidant

activity of HWEL and to clarify the mechanism of action.

MATERIALS AND METHODS

Materials

Samples of mung bean (*Phaseolus radiatus*), adzuki bean (*Phaseolus aureus*), and black bean (*Glycine max*) were obtained from Tainan District Agriculture Improvement Station, Taiwan, while that of rice bean (*Phaseolus calcaratus*) was purchased from a Chinese herb store in Tainan.

Chemicals

L-(+)-Ascorbic acid (AA), thiobarbituric acid (TBA), xanthine, xanthine oxidase, cytochrome c and α tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of extracts

Each sample (100 g) was extracted with 1 L of boiling water for 1 hour. The extracts were filtered; the residue was re-extracted under the same conditions, and the combined filtrates were evaporated to dryness under vacuum and the yield of soluble constituents (mung bean, adzuki bean, black bean and rice bean) were 18.76, 21.80, 15.64 and 24.14%, respectively.

Animals

Male ICR mice (6 weeks old) were obtained from the animal center, National Cheng Kung University, Tainan.They were housed in an air-conditioned room at 22 ± 3 ℃, 55 ±5% humidity, and fed a standard laboratory diet and tap water throughout the investigation.

$FeCl_2$-ascorbic acid stimulated lipid peroxidation in mouse liver homogenate

A mixture containing 0.5 ml of liver homogenate, 0.1 ml of Tris-HCl buffer (pH 7.2), 0.05 ml of 0.1 mM ascorbic acid, 0.05 ml of 4 mM $FeCl_2$ and 0.05 ml of various concentrations of crude extracts, or α –tocopherol, were incubated for 1 hr at 37 ℃. After incubation, 9 ml of distilled water and 2 ml of 0.6% TBA were added and then shaken vigorously. The mixture was heated for 30 min in a boiling water bath (100 ℃). After cooling, 5 ml of *n*-butanol were added and the mixture was shaken vigorously. The *n*-butanol layer was separated by centrifugation at 1000 x g for 10 min. The absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents expect liver homogenate.

Cytochrome *c* test

Samples were dissolved in distilled water to 10 mg/ml, diluted with distilled water to various concentrations, and added to a solution containing 0.07 units/ml of xanthine oxidase, 100 μM xanthine, and 50 μM cytochrome *c*. Following 3min incubation at the room temperature, absorption was read at 550 nm.

RESULTS

Anti-lipid peroxidation activity

The inhibitory effect of different concentrations of HWEL on malondialdehyde (MDA) production in mouse liver homogenate, induced by $FeCl_2$-ascorbic acid *in vitro*, are shown in Table 1. Inhibition of MDA formation increased with increasing concentrations of HWEL. All extracts, in the concentration range of 0.3-3.0 mg/ml, showed anti-lipid peroxidation activities, and the inhibition rates were in the range of 51-100%. Significant differences (P <0.05) were found between the different concentrations of hot water extracts in various legumes. The 50% inhibitory concentration (IC_{50}) values ranged from 0.15~0.26 mg/ml in the thiobarbituric acid test. These results indicate that legumes displayed remarkable antioxidant activity.

Free radical scavenger activity

Enzymatic formation of superoxide anions was estimated by reduction of cytochrome *c*. Scavenging effects of different concentrations of all extracts on superoxide anions are shown in Table 2. The scavenging effect of HWEL on the superoxide anions increased with increasing concentrations of extracts. All extracts in concentration range of 0.1-10.0 mg/ml showed antioxidant activity, and the scavenging rates were in the range of 21-100% (P <0.05). In the cytochrome *c* test, the IC_{50} values ranged from 0.10~0.46 mg/ml, both mung bean and adzuki bean extracts show significant antioxidant effects (P <0.05), and a scavenging effect on superoxide anions.

Table 1. Inhibitory effects of different concentrations of HWEL on MDA production in mouse liver homogenate, induced by $FeCl_2$-ascorbic acid.

Samples	Concentration (mg/ml)	MDA[1] nmole/mg protein	Inhibition rate[2]	IC_{50} (mg/ml)
$FeCl_2$ - AA + saline	-	20.4 ± 0.02	-	
Normal (Control)	-	7.0 ± 0.05	-	
$FeCl_2$ - AA + samples				
Mung bean	0.3	12.7 ± 0.20	57.31^{b}	0.17
Adzuki bean	0.3	12.1 ± 0.30	61.79^{a}	0.15
Black bean	0.3	13.5 ± 0.36	51.20^{c}	0.26
Rice bean	0.3	12.8 ± 0.09	56.49^{b}	0.17
$FeCl_2$ - AA + samples				
Mung bean	1.0	8.3 ± 0.13	90.07^{a}	
Adzuki bean	1.0	9.3 ± 0.15	82.54^{b}	
Black bean	1.0	9.8 ± 0.25	79.00^{c}	
Rice bean	1.0	8.5 ± 0.03	89.60^{a}	
$FeCl_2$ - AA + samples				
Mung bean	3.0	6.8 ± 0.10	100.00^{a}	
Adzuki bean	3.0	6.9 ± 0.23	100.00^{a}	
Black bean	3.0	7.5 ± 0.03	96.30^{bc}	
Rice bean	3.0	7.3 ± 0.10	97.61^{b}	
α-tocopherol	3.0	7.2 ± 0.03	98.30^{b}	

[1] MDA data are presented as the means ±S.D. (n = 6).

[2] The inhibitory rates within a column with different superscript letters are significantly different at $P < 0.05$.

Table 2. Superoxide scavenger activities of different concentrations of HWEL in the cytochrome c test[1]

Samples	Concentration (mg/ml)	Scavenging effect[2] (%)	IC_{50} (mg/ml)
Mung bean	0.1	42.35[b]	0.14
Adzuki bean	0.1	50.00[a]	0.10
Black bean	0.1	25.38[c]	0.30
Rice bean	0.1	21.40[d]	0.46
Mung bean	0.5	69.61[b]	
Adzuki bean	0.5	71.48[a]	
Black bean	0.5	60.70[c]	
Rice bean	0.5	50.25[d]	
Mung bean	1.0	92.84[a]	
Adzuki bean	1.0	92.60[a]	
Black bean	1.0	82.10[b]	
Rice bean	1.0	71.42[c]	
Mung bean	3.0	100.00[a]	
Adzuki bean	3.0	100.00[a]	
Black bean	3.0	92.60[b]	
Rice bean	3.0	78.13[c]	
Mung bean	5.0	100.00[a]	
Adzuki bean	5.0	100.00[a]	
Black bean	5.0	96.30[b]	
Rice bean	5.0	90.50[c]	
α –tocopherol	5.0	100.00[a]	

[1] Data are presented as the percentage scavenging of free radicals (n = 3).

[2] Values within a column with different superscript letters are significantly different at $P < 0.05$.