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Antioxidant and free radical-scavenging activities of seeds and agri-wastes of some varieties of soybean (*Glycine max*)

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Abstract

In order to find antioxidant potential, seeds of 30 varieties of *Glycine max* were studied for their total phenolic contents (TPC), flavonoids and antioxidant activity (AOA). The seed extracts showed wide variation of TPC from 6.4 to 81.7 mg GAE/g, flavonoids 3.5 to 44.6 mg QE/g and AOA 7.5% to 74.7%. Free radical-scavenging activity (FRSA), assayed by DPPH in terms of IC_{50} (inhibitory concentration), ranged from 0.14 to 0.80 mg/ml, EC_{50} (efficiency concentration) from 6.1 to 34.8 mg/mg DPPH, ARP (anti-radical power) 2.9 to 16.4 and reducing power from 1.9 to 4.7 ASE/ml. Variety Kalitur showed highest the FRSA followed by Alankar and Hara soya, as evident from their low IC_{50}, EC_{50} and high ARP values. Alankar, Kalitur NRC-37, PK-472, VLS-47, Hara soya varieties were with comparatively higher TPC (52.7–81.7 mg GAE/g), AOA (50.5–74.7%) and showed better inhibition of peroxide formation assayed through ammonium thiocyanate and egg yolk, non-site-specific and site-specific inhibition of hydroxyl radical induced deoxyribose degradation and ferrous ion-chelating capacity than did the other varieties. Seed extracts of these varieties and leaves of Kalitur showed significant protection against DNA damage caused by free radicals. The agri-wastes of some promising varieties, e.g. Alankar, Kalitur, NRC-37 and PK-472, showed TPC ranging from 27.4 to 167 mg GAE/g, total flavanoids from 10.4 to 63.8 mg QE/g and AOA from 26.5% to 84.7% and their values were highest in the leaves, followed by pod pericarp and twigs. Out of all the varieties studied, leaves of Alankar and Kalitur varieties were more potent free radical-scavengers than were seeds, pod pericarp or twigs. The specific phenolic compositions and their quantifications were performed by HPLC and MS/MS, which showed that the seeds of Kalitur were higher in genistin (127 μg/g), seeds and leaves of Alankar in diadzin (113 μg/g) and gallic acid (87.2 μg/g), respectively. The present studies may be of importance in varietal improvement, nutraceuticals, bio-pharmaceuticals and utilization of agri-wastes as possible cost-effective natural antioxidants.

Keywords: *Glycine max*; Phenols; Antioxidant activity; Free radical-scavenging activity; Anti-radical power; Reducing capacity; Chelating effect; DNA damage

1. Introduction

Soybean (*Glycine max* L.), an important legume, has a high protein content with nutritionally balanced amino acid profile. Its consumption may be associated with reduced risk of hyperlipidemia, cardiovascular diseases, osteoporosis, breast, prostate and colon cancers (Andlauer, Martena, & Furst, 1999; Bajpai, Sharma, & Gupta, 2005). It has various biologically active phytochemicals, e.g. isoflavones, coumestrol, phytate, saponins, lecithin, phytosterols and vitamin E, that provide several health benefits, including protection against oxidative stress (Tripathi & Misra, 2005). Phytoestrogens are known to have estrogenic, anti-carcinogenic, antiviral, antifungal, anti-osteoporotic and antioxidant activities. Saponins and phytate have been reported to show antioxidant and potential anti-carcinogenic properties (Fritz, Seppanen, Kurzer, & Csallany, 2003; Mazur, Duke, Wahäla, Rasku, & Adlercreutz, 1998).

Free radicals are able to oxidize biomolecules, leading to mutagenic changes, tissue damage and cell death (Yang,

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Mau, Ko, & Huang, 2000). They play a significant pathological role in cancer, emphysema, cirrhosis, atherosclerosis, arthritis and various other degenerative diseases. Certain phytochemicals, e.g. flavonoids, carotenoids, terpenoids, vitamins C and E and polyphenols, have been reported as promising antioxidants (Fritz et al., 2003; Patricia et al., 2005), and can reduce the access of oxidants and other deleterious molecules due to their ability to quench oxygen-derived free radicals by donating hydrogen atom or an electron, to chelate redox-active metals and inhibit lipooxygenases (Patricia et al., 2005; Tripathi & Misra, 2005). There is strong evidence that additive and synergistic interactions of natural antioxidants significantly strengthen the protective effects against oxidative damage. Soybean and its products are sources of important isoflavones, glycitein, genistein and daidzein that exert antioxidant action (Fritz et al., 2003; Andlauer et al., 1999) and soy may be treated as a consummate functional food because of its innumerable desirable characteristics.

The safety and toxicity of synthetic antioxidants have been important concerns; therefore, attention has been focussed on the use of natural antioxidants for inhibition or protection from oxidative damage. Therefore, the present studies were conducted to evaluate total phenolic contents, antioxidant potential, free radical-scavenging activity, metal-chelating capacity and their variation in seeds, and agri-wastes of some varieties of soybean for their possible utilization in foods or functional foods or pharmaceutical supplements.

2. Materials and methods

2.1. Materials and chemicals

The linoleic acid and β-carotene were purchased from Acros, USA; DPPH and authentic standards were from Sigma–Aldrich, USA; HPLC solvents and all other reagents of analytical grade were from E. Merck, India. Seeds and under-utilized parts of 30 varieties of *Glycine max,* cultivated under uniform cultural conditions at the National Research Centre for Soybean, Indore (India), were procured, dried, powdered (40-mesh) and stored in polythene bags. All the varieties were of pale yellow seed colour except for varieties Kalitur and Hara soya, which had black and green seed colour, respectively.

The powdered plant material (1.0 g) was extracted with 50% MeOH:H_2O (2 × 20 ml), overnight at room temperature and solvent from the combined extract was removed under reduced pressure. The total phenolic contents (TPC) in the extracts were measured by the method of Ragazzi and Veronese (1973) and were expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoids were estimated as described by Oyaizu (1986) and expressed as mg quercetin equivalents (QE)/g extract. The antioxidant activity (AOA) of extracts was determined by auto oxidation of a β-carotene and linoleic acid coupled reaction, according to Emmons and Peterson (1999), and was expressed as percent inhibition relative to control. Free radical-scavenging activity (FRSA) was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical according to Yen and Duh (1994) and the inhibitory concentration (IC_{50}), efficiency concentration (EC_{50}) and anti-radical power (ARP) were estimated and calculated as described by Kroyer (2004). Reducing capacity of extracts was determined (ASE/ml = absorbance of 1 mM ascorbic acid/absorbance of 1 mg/ml sample) by ferric reducing – antioxidant power assay (Apati et al., 2003) using quercetin as reference standard and expressed as ascorbic acid equivalents (1 mM = 1 ASE). Inhibition of lipid peroxidation was determined by using ammonium thiocyanate (Lee, Kim, Kim, & Jang, 2002) and egg yolk (Ohkowa, Ohisi, & Yagi, 1979). Hydroxyl radical-scavenging activity was measured (Halliwell, Gutteridge, & Aruoma, 1987) and the degree of both site-specific and non-site-specific deoxyribose oxidation was analysed as thiobarbituric acid-reactive species (TBARS). Ferrous ion-chelating capacity was estimated as described by Decker and Welch (1990). DNA nicking assay were performed, using supercoiled pUC 18 DNA, by the method of Lee et al. (2002) and analyzed on 1% agarose gel. For qualitative and quantitative analyses of phenols, samples were processed and studied as reported by Prakash, Singh, and Upadhyay (in press) by HPLC (Shimadzu LC-10A, Kyoto, Japan) and by MS/MS (API 2000, triple quadrupole mass spectrometer, Applied Biosystems, Ont., Canada).

3. Results and discussion

To find antioxidant potential, seeds from 30 varieties of *Glycine max* were studied (Table 1) for their total phenolic contents (TPC), flavonoids and antioxidant activity (AOA). TPC showed wide variation from 6.4 (Palam soya) to 81.7 mg GAE/g of seed extract (Kalitur, black seed colour), flavonoids from 3.2 (Palam soya) to 44.6 mg QE/g of extract (Alankar) and AOA, measured by auto oxidation of β-carotene and linoleic acid coupled reaction, from 7.5% (Palam soya) to 74.7% (Kalitur) in seed extracts of different varieties. Phenolic contents ranging from 4.54 to 8.09 mg catechin equiv./g of soy seed powder in 17 cultivars (Lee, Renita, St. Martin, Schwartz, & Vodovotz, 2004) and AOA, from 37.8% to 48.9% in Brazilian varieties of soybean, assayed by the same method (Genovese, Hassimotto, & Lajolo, 2005) have been reported. Depending on the quantitative and qualitative composition of antioxidants, different varieties of legume seeds can display different antioxidant activities (Troszyńska & Ciska, 2002). The isoflavone concentrations in soybean seeds comprise about 72% of the total phenols but are affected by various genotypic and environmental factors (Lee et al., 2003).

Varieties, Alankar, Kalitur, NRC-37, PK-472, VLS-47, of Hara soya (green seed colour) were found to have better amounts of TPC (52.7–81.7 mg GAE/g) that might be responsible for their comparatively higher antioxidant activity (50.5–74.7%). In some cases the AOA was high

Table 1
Total phenolic contents (TPC, mg/g of seed extract) expressed as gallic acid equivalents (GAE), antioxidant activity (AOA %) measured by auto oxidation of β-carotene and linoleic acid coupled reaction and flavonoid contents (mg/g of seed extract) expressed as quercetin equivalents (QE) of some varieties of *Glycine max*

Variety	TPC (mg/g of extract)	Flavanoids (mg/g of extract)	AOA (%)
Alankar	65.3	44.6	62.5
Hara Soya	52.7	26.2	50.5
Indira Soya	46.5	29.6	47.1
JS-75-46	36.3	14.7	31.1
JS-80-21	17.6	8.6	15.8
JS-97-52	24.7	20.5	7.9
JS-355	9.8	6.2	11.2
JS-335	7.6	4.9	28.5
Kalitur	81.7	42.9	74.7
KB-79	37.3	14.3	33.6
Lsb-1	24.8	11.8	18.9
Macs-13	39.6	16.9	31.2
Macs-330	8.4	4.7	12.8
Macs-993	16.8	7.6	14.9
Maus-61-2	17.6	8.7	20.7
Maus-71	16.9	10.5	21.6
Maus-47	31.4	19.2	27.1
Maus-81	49.5	27.3	48.2
NRC-2	48.7	27.2	45.0
NRC-37	57.2	31.9	55.6
Palam Soya	6.4	3.2	7.5
PK-327	42.5	19.5	27.1
PK-472	58.6	24.7	57.2
PS-1024	40.7	21.5	42.6
PS-1042	45.8	23.4	45.5
PS-1092	38.6	28.9	25.8
Pusa-16	40.3	17.1	38.7
Pusa-40	32.4	12.7	26.4
Raus-5	27.8	12.5	21.9
VLS-47	56.9	21.7	52.4
LSD at $P < 0.01$	1.83	2.53	1.18

in spite of low levels of TPC, and this might be due to the presence of some other antioxidant phytochemicals. Black soybeans showed better antioxidant capacity than did yellow soybeans, possibly due to the higher amounts of polyphenols, 29.0 and 0.45 mg/g, respectively (Takahasi et al., 2005). These results are in agreement with previous studies, where a correlation between the level of phenolic compounds and seed coat colour has been reported (Kim et al., 2006; Kim, Zheng, & Matsue, in press).

Phenols and flavonoids are known to be responsible for free radical-scavenging activity (FRSA). Promising varieties with high TPC and AOA were further subjected to FRSA, assayed by DPPH free radical, that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule and activity was expressed (Table 2) in terms of IC_{50} (inhibitory concentration) that ranged from 0.14 to 0.80 mg/ml, EC_{50} (efficiency concentration), ranging from 6.1 to 34.8 mg/mg DPPH and ARP (anti-radical power) from 2.9 to 16.4. Kalitur, the black coloured soybean variety, showed the highest FRSA, followed by Alankar and Hara soya (green coloured variety), as evident from their

Table 2
Free radical-scavenging activity (FRSA) of the seed extracts of some *Glycine max* varieties, measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in terms of IC_{50}, inhibitory concentration (mg/ml of extract)

Variety	IC_{50}	EC_{50}	ARP	ASE (ml)
Alankar	0.20	8.7	11.5	2.3
Hara Soya	0.23	10.6	9.1	3.1
Indira Soya	0.32	13.9	7.3	4.5
Kalitur	0.14	6.1	16.4	1.9
Maus-81	0.29	12.7	7.9	3.2
NRC-2	0.80	34.8	2.9	4.7
NRC-37	0.25	10.8	8.6	3.2
PK-472	0.28	12.4	8.2	3.6
PS-1042	0.37	16.1	6.2	2.9
VLS-47	0.33	14.3	6.9	3.4
Tea leaves	0.068	2.95	33.8	2.4
LSD at $P < 0.01$	1.84	5.68	2.92	1.20

EC_{50}, efficiency concentration (mg/mg DPPH); ARP, anti-radical power and reducing power (ASE/ml).
$EC_{50} = IC_{50}$/concentration of DPPH in mg/ml; ARP = 100/EC_{50}; ASE, ferric reducing antioxidant power expressed as ascorbic acid equivalents (1 mM = 1 ASE), which is inversely proportional to reducing power.

lower IC_{50}, EC_{50} and high ARP values, than the rest of the varieties. Soybean seed extracts tested for their H-donor ability, measured by DPPH, showed an IC_{50} value of 162 μg/ml (Georgetti, Casagrande, Vicenti, Verri, & Fonseca, 2006). The reducing power, expressed as ascorbic acid equivalents (ASE/ml), varied from 1.9 to 4.7 ASE/ml, which indicates their potential as electron donors to scavenge free radicals. The reducing capacities (Table 2) of Kalitur (1.9 ASE/ml) and NRC-37 (2.3 ASE/ml) were found to be better than that of green tea leaves (2.4 ASE/ml) that were used as a reference standard. However, all other varieties exhibited low efficiency as free radical scavengers.

The antioxidant activities of these varieties were further substantiated by inhibition of lipid peroxidation assayed through ammonium thiocyanate (Fig. 1a) and egg yolk (Fig. 1b). Kalitur, Alankar, NRC-37, VLS-47, Hara Soya, PK-472 showed better inhibition of peroxide formation than did the rest of the varieties. These varieties also showed better non-site-specific (Fig. 1c) and site-specific (Fig. 1d) inhibition of hydroxyl radical-induced deoxyribose degradation. The non-site-specific scavengers would compete with deoxyribose for the availability of hydroxyl radicals, resulting in a reduction of rate of the reaction. On the other hand, site-specific scavengers would offer protection by chelating with ferrous ions. It has been reported that extract of soybean with black seed coat inhibits LDL oxidation more significantly than does yellow seed coat (Takahasi et al., 2005) and soy phytochemicals exhibit antioxidant effects which include inhibition of lipid peroxidation, coupled oxidation of β-carotene and linoleic acid and effective hydrogen peroxide-scavenging activity (Kerry & Abbey, 1998; Fritz et al., 2003; Record, Dreosti, & Mc Inerney, 1995). Ferrous ion-chelating capacity (Fig. 1e) of Kalitur and Alankar was also comparatively higher than those of the other varieties. The results are consistent with

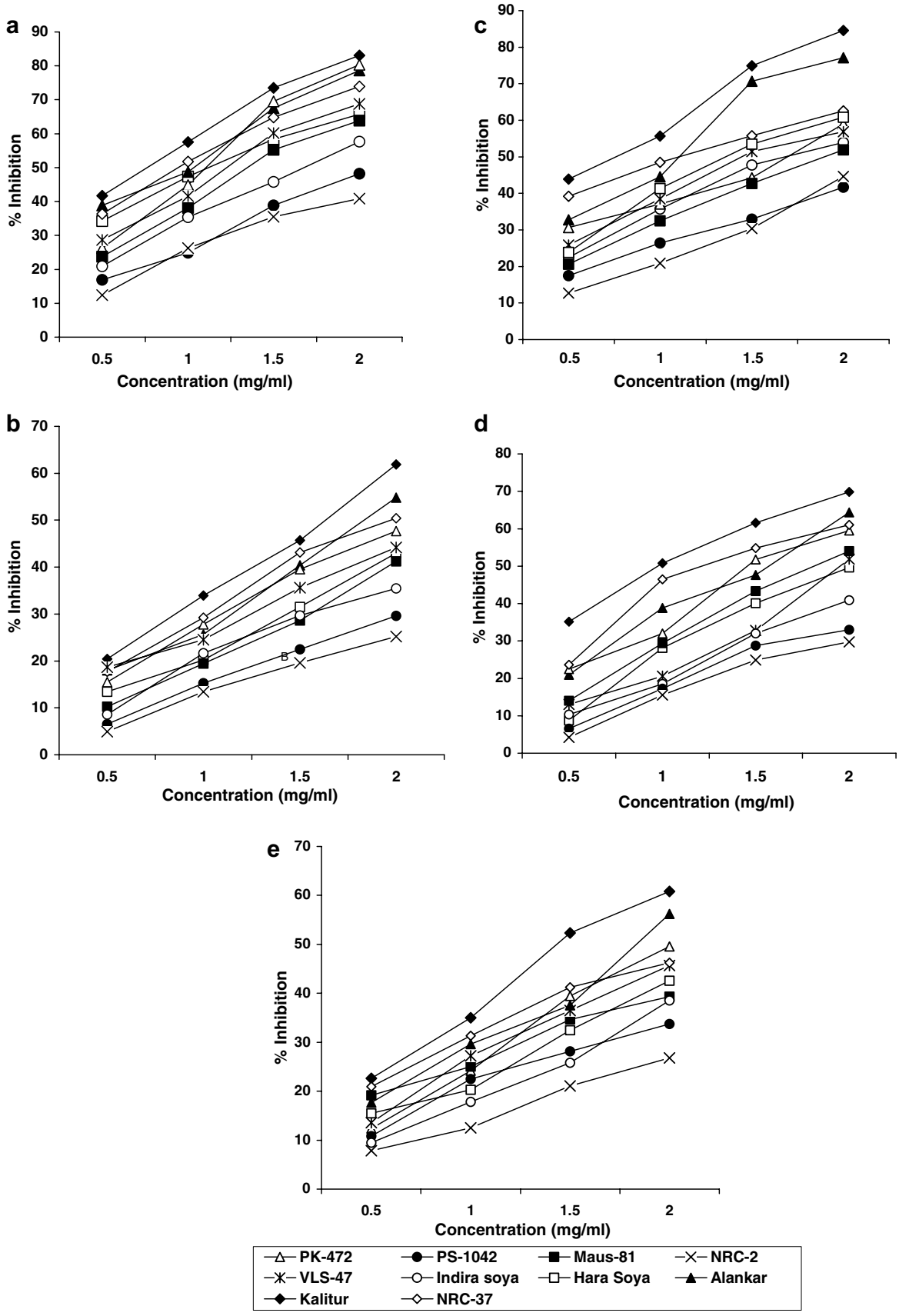

Fig. 1. Effect of seed extracts of different soybean varieties: (a) inhibition of lipid peroxidation assayed by ammonium thiocyanate method; (b) inhibition of lipid peroxidation using egg yolk; (c) non-site-specific inhibition of hydroxyl radical-mediated deoxyribose degradation; (d) site-specific inhibition of hydroxyl radical-mediated deoxyribose degradation; (e) ferrous ion-chelating capacity.

the previous report that phenolic compounds confer the high antioxidant activity of coloured grains (Kim et al., 2006; Kim, Zheng, et al., in press).

The agri-wastes, such as leaves, twigs and pod pericarp of some promising varieties (Alankar, Kalitur, NRC-37 and PK-472), were also investigated for their TPC, flavonoids, AOA and FRSA. The TPC varied from 27.4 to 167 mg GAE/g of total flavonoids from 10.4 to 63.8 mg QE /g and AOA from 13.2% to 84.7% (Table 3). In general, the amounts of TPC (98.6–167 mg GAE/g), flavonoids (39.7–63.8 mg QE/g) and AOA (69.8–84.7%) were higher in the leaves, followed by pod pericarp and twigs. These under-utilized parts were further tested for their FRSA (Table 4) and showed IC_{50} from 0.09 to 0.74 mg/ml, EC_{50} from 3.9 to 32.2 mg/mg DPPH, ARP from 3.1 to 25.1 and reducing power from 1.5 to 5.7 ASE/ml. The leaves of Alankar and Kalitur varieties were more potent as free radical-scavengers than were seeds (Table 2), pod pericarp and twigs (Table 4) among the varieties studied.

The concentration-dependent (5–20 μg/ml) scavenging effects of Kalitur seed extract on Fe^{3+}-induced free hydroxyl radicals showed protection against DNA damage (Fig. 2a) and mitigated the oxidative stress. Seed extracts of Alankar, NRC-37 and PK-472, at 20.0 μg/ml, showed significant reduction in the formation of nicked DNA (form II, circular) and increased native (form I, supercoiled) DNA (Figs. 2a and 2b). Further, *in vitro* protection against DNA damage by extracts of leaves, twigs and pod pericarp of Kalitur variety showed that the leaves were

Table 3
Total phenolic contents (TPC, mg/g extract), expressed as gallic acid equivalents (GAE), antioxidant activity (AOA %) and flavonoid contents (mg/g extract), expressed as quercetin equivalents (QE), of agri-wastes of some selected varieties of *Glycine max*

Variety	Parts	TPC (mg/g) extract	Flavanoids (mg/g) extract	AOA (%)
Alankar	Leaves	167	63.8	84.7
	Pod pericarp	52.0	21.7	38.4
	Twigs	34.6	10.4	16.5
Kalitur	Leaves	151	60.3	80.9
	Pod pericarp	59.5	32.7	44.2
	Twigs	38.7	15.8	20.1
NRC-37	Leaves	98.6	39.7	69.8
	Pod pericarp	42.4	24.1	37.4
	Twigs	31.6	12.6	13.2
PK-472	Leaves	107	42.2	72.5
	Pod pericarp	48.1	20.8	31.3
	Twigs	27.4	12.1	14.5
For varieties	LSD at $P < 0.01$	2.53	0.61	2.01
For parts	LSD at $P < 0.01$	1.41	1.26	1.32

Table 4
Free radical-scavenging activity (FRSA), measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in terms of IC_{50} = inhibitory concentration (mg/ml of extract)

Variety	Parts	IC_{50}	EC_{50}	ARP	ASE (ml)
Alankar	Leaves	0.09	3.9	25.1	1.5
	Pod pericarp	0.26	11.6	8.6	3.2
	Twigs	0.68	29.8	3.4	5.7
Kalitur	Leaves	0.12	5.0	20.0	1.9
	Pod pericarp	0.22	8.3	12.1	2.7
	Twigs	0.60	26.3	3.8	5.1
NRC-37	Leaves	0.20	8.7	11.4	2.1
	Pod pericarp	0.37	16.1	6.2	3.5
	Twigs	0.71	31.0	3.2	5.3
PK-472	Leaves	0.16	6.9	14.5	2.3
	Pod pericarp	0.29	12.8	7.8	4.1
	Twigs	0.74	32.2	3.1	4.8
LSD at $P < 0.01$	For varieties	0.03	1.05	2.62	1.59
LSD at $P < 0.01$	For parts	0.02	0.98	1.66	1.27

EC_{50} = efficiency concentration (mg/mg DPPH); ARP = anti-radical power and reducing power (ASE/ml) of agri-wastes of some selected varieties of *Glycine max*.

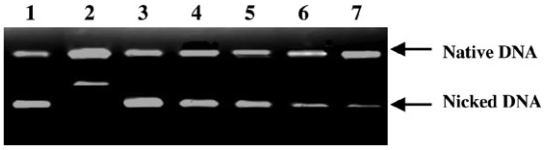

Fig. 2a. Concentration-dependent inhibitory effects of Kalitur seed extract on native pUC18 DNA, nicking caused by hydroxyl radicals. Lane 1: pUC18 DNA; Lane 2: DNA + Fenton; Lane 3: DNA + Fenton + SOD (2U); Lane 4: DNA + Fenton + 20 μg/ml; Lane 5: DNA + Fenton + 15 μg/ml; Lane 6: DNA + Fenton + 10 μg/ml; Lane 7: DNA + Fenton + 5.0 μg/ml.

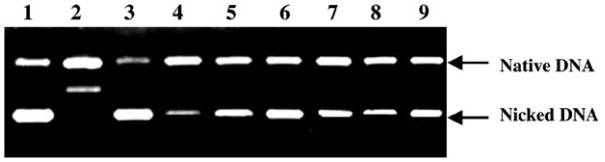

Fig. 2b. Inhibitory effects, using soy seed extracts (20 μg/ml) of some promising varieties on native pUC18 DNA, nicking caused by hydroxyl radicals. Lane 1: pUC18 DNA; Lane 2: DNA + Fenton; Lane 3: DNA + Fenton + SOD(2U); Lane 4: DNA + Fenton + PS-1042; Lane 5: DNA + Fenton + PK-472; Lane 6: DNA + Fenton + Alankar; Lane 7: DNA + Fenton + NRC-37; Lane 8: DNA + Fenton + Hara Soya; Lane 9: DNA + Fenton + VLS-47.

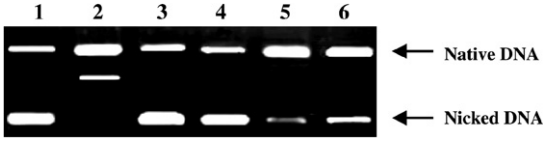

Fig. 2c. Inhibitory effect of under-utilized parts (20 μg/ml) of Kalitur variety of soybean on native pUC18 DNA nicking caused by hydroxyl radicals. Lane 1: pUC18 DNA; Lane 2: DNA + Fenton; Lane 3: DNA + Fenton + SOD (2U); Lane 4: DNA + Fenton + Leaves; Lane 5: DNA + Fenton + Twigs; Lane 6: DNA + Fenton + Pod pericarp.

more efficient hydroxyl radical-scavengers than were pod pericarp and twigs (Fig. 2c). A decrease in oxidative DNA damage has been ascribed to isoflavones, genistein and daidzein in soy (Fritz et al., 2003); however, the combined effect of soy isoflavones might be better than the effect of any single iso-flavone compound (Georgetti et al., 2006). Transition metal ions are known to catalyze the formation of free radicals and reduction in the formations of single-stranded nicked DNA (form II, circular), double-stranded nicked DNA (form III, linear) and increased form I (supercoiled) DNA. Antioxidant effect of *Opuntia ficus-indica*, against oxidative DNA damage, has shown similar results at the same concentration (Lee et al., 2002). The most probable reason for their potential as free radical-scavengers and protection against DNA damage might be related to polyphenol and flavonoid contents as they have been reported to inhibit lipid peroxidation by scavenging reactive oxygen species, chemiluminescence reactions and tumorigenesis (Georgetti et al., 2006; Lee et al., 2005). They are also known as powerful protecting agents against the lethal effects of oxidative stress and offer protection of DNA by chelating redox-active transition metal ions. The present studies, together with the previous works, suggest the triple synergistic action of phenols in scavenging free radicals, repairing DNA and metal chelation (Lee et al., 2002).

Seeds of promising varieties were further examined for their specific phenolic composition by HPLC (Table 5) that showed the presence of phenolic acids and flavonoids. The quantities of gallic acid were ranging from 9.8 to 32.1 μg/g, chlorogenic acid 18.6 to 47.2 μg/g, caffeic acid 6.7 to 26.4 μg/g, ferulic acid 5.3 to 48.2 μg/g, rutin 5.1 to 31.3 μg/g, quercetin 4.6 to 18.2 μg/g, kaempferol 3.5 to 41.4 μg/g, daidzin 28.1 to 113 μg/g, genistin 40.8 to 127 μg/g, daidzein 4.1 to 31.7 μg/g and genistein 3.5 to 27.2 μg/g. Our results confirm the presence of gallic acid (0.25–1.00 μg/g), chlorogenic acid (2.4–23.3 μg/g), caffeic acid (0.97–12.8 μg/g), ferulic acid (1.82–23.5 μg/g), rutin (2.6–15.9 μg/g), kaempferol (0.39–1.56 μg/g) and quercetin (0.46–6.5 μg/g), as reported earlier in nine varieties of soybean (Kim et al., 2006; Kim, Zheng, et al., in press).

Among all the varieties studied, Kalitur was found to be the richest source of genistin (127 μg/g) and genistein (27.2 μg/g) whereas Alankar was richest in diadzin (113 μg/g) and daidzein (31.7 μg/g). Comparatively better amounts of gallic (32.1 μg/g) and ferulic (48.2 μg/g) acids were observed in Kalitur, chlorogenic acid (47.2 μg/g) in PK-472, rutin (31.3 μg/g) in Hara soya and kaempferol (41.4 μg/g) in VLS-47 variety. Quantities of daidzin 337–677 (μg/g), genistin 863–1401 (μg/g) and total isoflavone 1303–2015 (μg/g) were found in seeds of four soybean cultivars (Swanson, Stoll, Schapaugh, & Takemoto, 2004); daidzein and genistein, up to 3.8×10^6 μg/kg wet wt (Reinli & Block, 1996), have been reported earlier. Similarly, Achori, Boye, and Belanger (2005) found amounts of diadzin and genistin ranging from 558 to 770 μg/g and 883 to 1136 μg/g, respectively in soy meal from three different solvent systems. The soybean isoflavone composition showed a variation from 13.1 to 160 μg/g of diadzin, 41.5 to 240 μg/g of genistin, 1.1 to 43 μg/g of diadzein and 0.5 to 31.6 μg/g of genestein in eight soybean cultivars (Kim, Kim, Hahn, & Chung, 2005). Isoflavone contents in Chinese and Korean soybeans were: diadzin 152 and 259 μg/g, daidzein 3.5 and 6.5 μg/g, genistin 519 and 855 μg/g, genestein 1.4 and 6.3 μg/g, respectively (Kim, Zheng et al., in press). Accumulation of phenols is dependent on soil, cultivar and environmental conditions during the seed formation. Low temperatures during the onset and duration of seed fill have been shown to increase the isoflavone content in soybean several-fold (Georgetti et al., 2006). The phenolic profiles of the plants may be changed, depending on the conditions imposed by soil, season, climate, plant component and other parameters (Kim et al., 2006; Kim, Zheng, et al., in press).

The leaves and pod pericarp of varieties found to have better amounts of TPC were also examined for their specific phenolic composition (Table 6), and quantity of gallic acid varied from 16.4 to 87.2 μg/g, chlorogenic acid 27.4 to 45.6 μg/g, ferulic acid 9.7 to 61.3 μg/g, rutin 35.6 to 59.5 μg/g, quercetin 4.7 to 31.7 μg/g, kaempferol from 7.5 to 27.7 μg/g, daidzein 0.3 to 7.2 μg/g, and genistein 0.9 to 8.7 μg/g of plant material. Pod pericarp did not show the

Table 5
Specific phenolic composition (μg/g seed powder) in seeds of some varieties of *Glycine max*

	Alankar	Hara Soya	Indira Soya	Kalitur	Maus-81	NRC-2	NRC-37	PK-472	PS-1042	VLS-47
GA	26.2	14.2	12.1	32.1	18.7	9.8	27.9	21.7	12.8	24.6
FA	42.5	40.6	25.3	48.2	19.7	14.2	28.4	36.2	5.3	27.7
ChA	41.2	37.4	26.9	38.5	40.8	28.2	34.3	47.2	18.6	30.9
RT	26.2	31.3	13.7	29.4	20.4	5.1	16.4	24.7	7.2	19.5
CA	18.7	10.4	13.6	11.9	12.4	7.1	26.4	17.3	6.7	14.5
QC	8.2	12.4	4.6	18.2	7.9	5.7	11.9	16.2	14.4	17.6
Kmp	33.1	21.7	17.2	31.9	24.8	8.5	34.7	30.2	3.5	41.4
Dzin	113	51.7	43.6	93.1	64.8	34.4	58.7	62.5	28.1	51.4
Gstin	85.9	96.4	52.3	127	71.6	40.8	79.9	91.3	51.2	74.9
Dzein	31.7	12.8	5.7	18.5	10.4	4.1	17.3	22.6	7.5	6.9
Gstein	21.4	6.3	4.8	27.2	11.9	3.5	24.1	18.4	5.1	15.5

GA, gallic acid; ChA, chlorogenic acid; CA, caffeic acid; EA, ellagic acid; FA, ferulic acid, Kmp, kaempferol; QC, quercetin; RT, rutin; Dzin, daidzin; Gstin, genistin; Dzein, diadzein; Gstein, Genestein.

Table 6
Specific phenolic composition (μg/g of plant material) in leaves and pod pericarp of some varieties of *Glycine max*

	Kalitur		Alankar		NRC-37		PK-427	
	Leaves	Pod pericarp	Leaves	Pod pericarp	Leaves	Pod pericarp	Leaves	Pod pericarp
GA	80.5	30.2	87.2	19.2	85.7	16.4	67.4	26.5
ChA	40.4	–	36.6	–	45.6	–	27.4	–
RT	59.5	–	42.1	–	46.3	–	35.6	–
FA	52.8	19.4	61.3	18.7	41.5	9.7	34.9	13.9
Dzein	6.5	1.7	7.2	0.8	2.9	0.3	5.8	1.7
QC	26.8	7.6	14.3	6.4	21.0	5.2	31.7	4.7
Kmp	27.7	13.5	10.8	22.1	21.7	15.9	24.9	7.5
Gstein	8.2	1.2	5.6	1.8	4.0	0.9	6.5	1.4

GA, gallic acid; ChA, chlorogenic acid; FA, ferulic acid; Dzein, diadzein; Kmp, kaempferol; QC, quercetin; RT, rutin; Gstein, genistein.

Table 7
Phenolic composition of some *Glycine max* varieties identified by MS/MS

Phenols	Ion full scan MS		MS/MS approach
	$[M-H]^-$	Fragments	Product ion scan
Gallic acid	169	125	169
Chlorogenic acid	353	191	353
Caffeic acid	179	135	179
Ferulic acid	193	178, 134	193
Diadzein	253	134, 151, 167, 180, 195, 208, 223	132
Kaempferol	285	133, 151	285
Quercetin	301	151	301
Rutin	609	301	609
Genistein	269	117, 124, 151, 168, 180, 195, 238	269
Genistin	432	117, 151, 168, 195, 224, 238	432
Daidzin	416	134, 167, 195, 208, 223	416

presence of rutin or ferulic acid. The tissue-specific differences in the types and contents of phenolic compounds are dependent on the soybean varieties (Kim et al., 2006; Kim, Zheng, et al., in press).

The identification of specific polyphenols in seeds, leaves and pod pericarp was further substantiated by MS/MS analysis (Table 7), that showed the deprotonated molecule $[M-H]^-$. Loss of CO_2 was observed for caffeic, gallic and protocatechuic acids, giving $[M-H-44]^-$ as a characteristic ion. Chlorogenic acid showed the deprotonated molecule $[M-H]^-$ at m/z 353 and ion corresponding to the deprotonated quinic acid at m/z 191. Flavonol *O*-glycosides, such as rutin, diadzin, genistin, showed the deprotonated molecule of the glycoside and ion corresponding to the deprotonated aglycone $[A-H]^-$. The latter ions were formed by loss of rutinose in the case of rutin whereas diadzin and genistin, showed loss of a glucose moiety. Finally the aglycones, such as quercetin, kaempferol and genestein, gave retro-Diels–Alder fragmentations where m/z 151 was common, but, in the case of daidzein, m/z at 134 was observed.

The identified phenolic compounds have well-documented free radical- scavenging activities and metal ion-chelating capacity. Gallic acid, present in soybean, especially in leaves, has been reported (Sanchez-Moreno, Larrauri, & Saura-Calixto, 1999) with high free radical-scavenging capacity compared to rutin, ferulic acid, caffeic acid and BHA. The soybean seeds are rich sources of flavonoids, such as daidzein and genistein and, even the under-utilized parts, were found to have reasonable amounts. In soybean, isoflavones were principally found in seeds; however, the metabolites were also isolated from leaf and stem tissues (Bennett, Oliver, Heatherly, & Krishnan, 2004). It has been found that plant extracts containing flavonoids and chlorogenic acid are highly effective in scavenging DPPH radical (Apati et al., 2003), hydroxyl radical and in metal-chelating capacity (Lean et al., 1999). Similarly, the mechanism of action of quercetin also includes free radical-scavenging, chelation of metal ions and inhibition of lipid peroxidation (Dušinská et al., 1999). The appreciable concentrations of flavonoids, along with phenolic acids and other antioxidant phytochemicals present in different soybean cultivars and their agri-wastes under study, might be responsible for their efficient free radical-scavenging activity. Reactive oxygen species can cause damage to cellular biomolecules, e.g. DNA, RNA, enzymes, lipids and carbohydrates, and consequently may adversely affect immune functions. Oxidation of bases in DNA, deoxyribose lesions and strand breaks may lead to mutagenic changes and a variety of diseases (Dušinská et al., 1999; Lee et al., 2002). Phenols, due to their strong antioxidant and (a range of) biological properties are also known to diffuse the toxic free radicals (Bingham et al., 2003; Hertog, Feskens, Hollman, Katan, & Kromhout, 1993).

From the foregoing it may be concluded that studies on intervarietal variation, in soybean seeds, of phenolic contents, antioxidant and free radical-scavenger activities may be of importance in varietal improvement, nutraceuticals and bio-pharmaceuticals and utilization of agri-wastes as possible cost-effective natural antioxidants.

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