



Bioprospecting nutraceuticals from soybean (*Glycine max*) seed coats and cotyledons

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ABSTRACT

Food security coupled with nutritional security is a great concern to address the menace of malnutrition. In the present study, total phenolic contents and antioxidant potential of 35 soybean genotypes have been determined (2018). Besides, the solvent system for efficient extraction of total phenolic content coupled with antioxidants (nutraceuticals) has been optimized. The results revealed that the higher total phenolic contents from soybean seed coats and cotyledons were obtained in acetone-water-acetic acid (70:28:02, v/v) mixture. Total phenolic content (TPC) in soybean genotypes were in the range of 2.58–51.37 µg/mL and 4.26–12.76 mg/mL in seed coats and cotyledons, respectively. In soybean seed coats, higher phenolic content was observed in JS76-205 genotype with 128.5 µg/ml, while JS-2 and MAUS-158 showed 9.00 µg/mL. On the other hand, TPC derived from soybean cotyledons of NRC-37 and MAU-81 showed 12.76 mg/mL; whereas in PS-1347 resulted 4.26 mg/mL. Characterization of phenolic compounds in soybean seed coat and cotyledon revealed the presence of protocatechuic acid, *p*-hydroxy benzoic acid, 4-hydroxy benzaldehyde, vanillic acid, vanillin, *p*-coumaric acid and ferulic acid. Further, antioxidant studies performed from soybean seed coats and cotyledons were in the range of 9.00–128.50 µg eq. ascorbic acid and 2.13–4.27 mg eq. ascorbic acid, respectively. This study demonstrates that the TPC derived from soybean coat and cotyledon can be used not only as nutraceutical but also ensure food and nutritional security.

Key words: Antioxidants, Nutraceuticals, Soybean, Total phenols

Burgeoning population, shrinkage of natural resources and climate changing conditions have jeopardized sustainable development goals. The United Nations report revealed that the world population would surge to 8.5 billion in 2030, which alarm to find alternative way for sustainable food production (Ripple *et al.* 2017, Sinha *et al.* 2017). Hence, to address these issues bioprospecting of natural resources for enriched nutrients coupled with quality antioxidants is a viable option. Among the natural enriched food sources, soybean is one of the predominant crops, which can satiate both the requirements of nutraceuticals. It is a leguminous crop that is widely grown in tropical, sub-tropical and

temperate regions around the world (Chandusingh *et al.* 2018, Singh *et al.* 2019). Besides, soybean possesses 35% protein, 25–30% carbohydrates and significant amount of polyunsaturated fatty acids (PUFAs), vitamins, minerals, antioxidants and quality fibre (Bueno *et al.* 2018). It is evenly popular for seed oil and cultivated in India at large scale for various uses such as edible oil, soy whey, tofu, textured soy protein (TSP), tempeh, soya sauce and miso. In 2017–18, the soybean production in India was 10 million tonnes, whose share is 2.9% of global soybean production (www.statista.com accessed on 5th May, 2018). In India, more than hundred varieties of soybean have been released, of which 32 genotypes (approx) were highly indented in soybean seed chain (Agarwal *et al.* 2013).

Studies pertaining to bioprospecting of soybean nutraceutical properties, very few reports have deciphered their biochemical composition with few soybean genotypes (Kumar *et al.* 2015). Moreover, total phenolics extraction from different soybean genotypes varies due to several features that need to be standardized from soybean seed coat and cotyledon. Hence, to address these issues studies was carried out with an aim to bioprospect the promising soybean genotype and optimize total phenolic contents (TPC) extraction deploying solvent systems from seed coat and cotyledon. In addition to TPC, antioxidants potential of

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various soybean genotypes has been determined.

MATERIALS AND METHODS

Seed material of 35 genotypes of soybean (PUSA-9712, Kalitur, MAUS-71, Harasoya, JS-2, JS-7105, RKS-18, NRC-7, VLS-47, JS-8031, TAMS-38, SL-525, MAUS-450, Bragg, PS-1347, PUSA-9814, MAUS-158, JS97-52, NRC-2, Shivalika, JS-335, PS-1225, JS76-205, JS-9041, MAUS-61, JS-2034, JS-9305, JS-2029, MAUS-81, JS-9560, PS-1042, NRC-37, JS-7546, RAUS-5 and PS-1241) was procured from Indian Institute of Soybean Research, Indore, Madhya Pradesh (2018). Different seeds of soybean genotypes were immersed in distilled water for 12 h in order to weaken the seed coat. The seed coat and cotyledon were separated gently and allowed to dry for 1 h at 40°C. After incubation, seed coats and cotyledons of each genotype were crushed in mortar and pestle with different solvent mixture followed by centrifugation of the samples at 5000 rpm for 5 min (Singh *et al.* 2015). The supernatant obtained was used for TPC and antioxidants determination. Different solvents employed in this study for TPC extraction were acetone (A), water (W) acetic acid (Aa), methanol (M) and ethanol (E). To enhance higher solubility of TPC, different solvent mixtures used were; AWAA-1 (70:28:02), AWAA-2 (70:29.5:0.5), AWAA-3 (70:29.8:0.2), MW-1 (50:50), MWAA-2 (50:49.5:0.5), MAa-3 (99.5:0.5) and EW (70:30), respectively.

Total phenolics estimation: Total phenolic content of different soybean genotypes was determined according to Xu *et al.* (2007). Assay was done by drawing 0.1 mL of seed coat and cotyledon extracts into 2.0 mL of Folin-Ciocalteu reagent (FCR) followed by addition of 0.9 mL of distilled water. After incubation for 5 min in dark chamber, 0.3 mL of 20% (w/v) sodium carbonate was added. The mixture was incubated at room temperature for 20 min and absorbance was measured at 765 nm, which was expressed as µg phenol/mL of TPC.

Antioxidant determination: The free radical scavenging activity in seed coat and cotyledon extracts of different genotypes was estimated using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay (Zlotek *et al.* 2016). DPPH (1 mM) solution was prepared in absolute ethanol. The seed coat and cotyledon extracts (100 µL) was added in 2 mL of DPPH solution and the reaction mixture was incubated for 5 min. The colour intensity developed during the reaction was measured at 525 nm against the blank. Percentage inhibition of free radical DPPH was estimated based on equation (1) and antioxidant potential was expressed in µg ascorbic acid equivalents (AAE)/g dry weight.

$$\text{Activity (\%)} = \left[\frac{A_{\text{con}} - B_{\text{test}}}{A_{\text{con}}} \right] \times 100 \quad (1)$$

where A_{con} , O.D of blank solution; B_{test} , O.D of samples.

Characterization of phenolics using high performance liquid chromatography (HPLC): The TPC presence in the soybean seed coat and cotyledon was extracted using alkaline hydrolysis according to Campbell and Ellis (1992). The sample preparation for HPLC analysis and the experimental

conditions for HPLC program were kept according to Ascenosao and Dubery (2003).

RESULTS AND DISCUSSION

Solvent system analysis: Total phenolic content derived from seed coats and cotyledons of kalitur and JS76-205 was higher than RAUS-05 and harasoya in acetone:water:acetic acid (70:28:2, WAA-1). Research results implied that acetone–water coupled with acetic acid has higher solubility of phenolic compounds than the other solvents system. Probable reason for higher TPC in AWAA-1 might be due to higher solvency of solvent mixture towards structural features of TPC (Kumar *et al.* 2017). Variation of TPC not only depends on solvent system but also on distribution among soybean genotypes (Alghamdi *et al.* 2018). Jeng *et al.* (2010) reported that soybean genotypes with black seed coat have high TPC, which is in good coherence with the present study.

Total phenolic content (TPC): Total phenolic content from 35 soybean genotypes was evaluated of which black seed coat genotypes kalitur and JS76-205 showed higher phenolic content of 128.50 µg/mL and 117.00 µg/mL, respectively. The green coated seed harasoya showed phenolic content of 14.30 µg/mL (Fig 1), whereas JS-2 and MAUS-158 resulted lowest TPC of 9 µg/mL. Among total phenolic contents evaluated in cotyledon of soybean genotypes, NRC-37 and MAUS-81 exhibited highest TPC with 12.76 mg/mL, while PS-1347 genotype resulted 4.26 mg/mL of TPC.

Antioxidant potential of soybean genotypes: Recent studies illustrate that the reactive oxygen species (ROS) is an important variable that determine the seed dormancy release, seed germination and seed death (Kumar *et al.* 2015, Sarangi *et al.* 2019). Neutralizing ROS from oxidative stress by antioxidants could be considered as seed quality marker (Kumar *et al.* 2016, Tiwari *et al.* 2018). Hence, in the study the TPC determined from the seed coats and cotyledons from soybean has been assessed for the antioxidant potential. Seed coat of different soybean genotypes kalitur and JS76-205 showed 51.38 and 46.07 µg eq. ascorbic acid, respectively. Lower antioxidant potential was recorded in white colored genotype RAUS-05 with 2.58 µg eq. ascorbic acid (Fig 2). In case of cotyledons, higher antioxidant potential was observed in kalitur with 4.27 mg eq. ascorbic acid; while JS97-52 showed 2.14 mg eq. ascorbic acid (Fig 2). In harasoya, the antioxidant potential in seed coat and cotyledon was 14.30 µg eq. ascorbic acid and 6.28 mg eq. ascorbic acid, respectively. The antioxidant potential governed by the phenolic compounds present in seed have significance role in the seed germination. The observed pattern of positive correlation with TPC and antioxidants is in good agreement with the studies (Baginsky *et al.* 2013). Research reports elucidate that the pigmented seed coats of soybean are rich in polyphenolic contents (Lee *et al.* 2009). The variation in seed coat colour of soybean is due to presence of phenolic compounds such as flavonoids and polyphenol oxidase enzyme, which convert phenolic

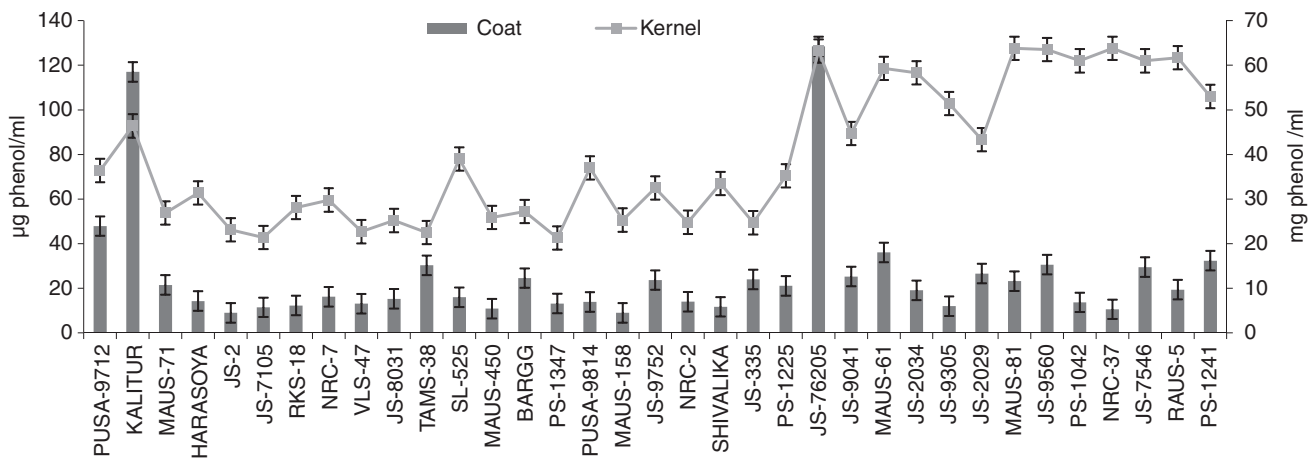


Fig 1 Distribution of total phenolic content in different soybean seed coats and kernels

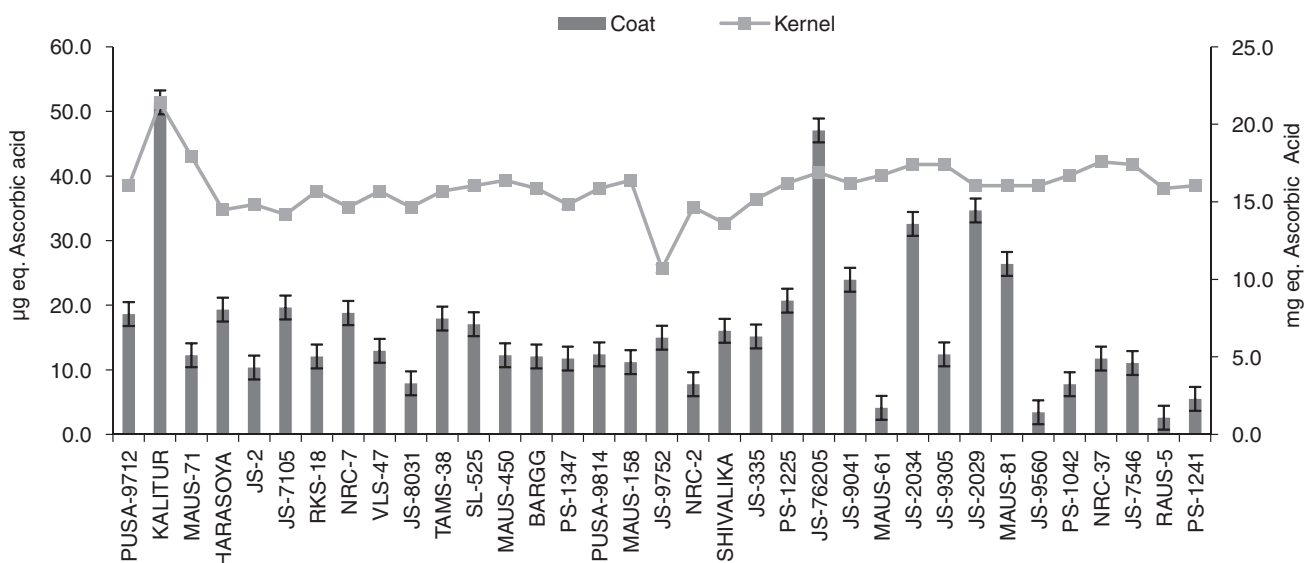


Fig 2 Antioxidant potential of different soybean seed coats and kernels

compounds to brown pigments by forming *o*-quinones (color pigmentation) during seed maturation (Chandusingh *et al.* 2017). Xu and Chang (2008) reported that the black seed coat has higher free radical scavenging activity than green and white seed coats.

Characterization of phenolics: Characterization of phenolic compounds derived from seed coats and cotyledons were determined using high performance liquid chromatography (HPLC). Studies indicate presence of compounds such as protocatechuic acid, *p*-coumaric acid, *t*-ferulic acid, vanillic acid, vanillin, 4-hydroxy benzoic acids and 4-hydroxy benzaldehyde. In soybean seed coat, presence of protocatechuic acid, 4-hydroxy benzaldehyde, 4-hydroxy benzoic acid and vanillin was found predominant in kalitur genotype with 3.41, 1.40, 0.12, and 0.06 µg/100 mg of dry weight tissue, respectively. However, in JS76-205 protocatechuic acid showed significantly lower content with 1.26 µg/100 mg of dry weight tissue. The genotype RAUS-5 showed lower antioxidant potential but higher concentration in vanillic acid, *p*-coumaric acid and *t*-ferulic acids with 0.56, 0.18 and 1.33 µg/100 mg of dry weight

tissue in seed coat, respectively (Table 1). Conversely in cotyledon of kalitur, protocatechuic acid, *p*-coumaric acid and vanillin were found predominantly (Table 1).

Several investigations emphasized that protocatechuic acid is an abundant polyphenol compound than anthocyanins in soybean seed coat ((Josipovic *et al.* 2016). It is an important antioxidant that has beneficial effect on human health and phenolics coupled with quality antioxidants exhibited positive effect on physiological activity that benefits in lowering the risk of cardiovascular diseases (Shumoy *et al.* 2017). Moreover, some other positive effects exerted by TPC are anti-inflammation, free radical scavenging activity, inhibition of peroxidation and estrogenic activity (Ayala *et al.* 2014). This study implies that the TPC coupled with antioxidant derived from soybean seed coat and cotyledon could serve as potential nutraceutical that ultimately address the problem of food-cum-nutritional security (Kumar *et al.* 2019). Besides protocatechuic acid, *p*-coumaric acid showed anti-hepatic and anti-nephrotoxic properties in rats. Akdemir *et al.* (2017) administered cisplatin compound (hepto and nephrotoxin) to the rats and

Table 1 Characterization of phenolics in seed coat and cotyledon of soybean genotypes

| Phenolics | Soybean seed coat | | | |
|-------------------------|-------------------|-----------------|-----------------|-----------------|
| | JS-76205 | Harasoya | Kalitur | RAUS-5 |
| Protocatechuic acid | 1.2623 ± 0.0931 | 0.0059 ± 0.0004 | 3.4114 ± 0.3112 | 0.0308 ± 0.0029 |
| 4-Hydroxy benzoic acid | 0.1076 ± 0.0105 | 0.0271 ± 0.0022 | 0.1169 ± 0.0108 | 0.1059 ± 0.0098 |
| Vanillic acid | 0.0611 ± 0.0061 | 0.4581 ± 0.0413 | 0.4343 ± 0.0425 | 0.5611 ± 0.0523 |
| 4-Hydroxy benzaldehyde | 0.9048 ± 0.0836 | 0.9827 ± 0.0923 | 1.3953 ± 0.1014 | 1.3711 ± 0.1132 |
| Vanillin | 0.0275 ± 0.0021 | 0.0229 ± 0.0019 | 0.0645 ± 0.0058 | 0.0279 ± 0.0023 |
| <i>p</i> -Coumaric acid | 0.0335 ± 0.0029 | 0.0918 ± 0.0083 | 0.1276 ± 0.0112 | 0.1810 ± 0.0168 |
| <i>t</i> -Ferulic acid | 0.0977 ± 0.0093 | 0.7457 ± 0.0718 | 0.8672 ± 0.0832 | 1.3259 ± 0.1214 |
| Phenolics | Soybean cotyledon | | | |
| | JS-76205 | Harasoya | Kalitur | RAUS-5 |
| Protocatechuic acid | 0.0439 ± 0.0036 | 0.0519 ± 0.005 | 1.307 ± 0.094 | 0.0208 ± 0.0029 |
| 4-Hydroxy benzoic acid | 0.0894 ± 0.0088 | 0.0342 ± 0.0031 | 0.202 ± 0.018 | 0.254 ± 0.022 |
| Vanillic acid | 0.4614 ± 0.0441 | 0.9702 ± 0.0936 | 0.638 ± 0.049 | 0.3611 ± 0.0523 |
| 4-Hydroxy benzaldehyde | 1.2331 ± 0.1211 | 2.3264 ± 0.2121 | 0.532 ± 0.046 | 0.3111 ± 0.1132 |
| Vanillin | 0.0251 ± 0.0024 | 0.0332 ± 0.0031 | 0.0645 ± 0.0058 | 0.0279 ± 0.0023 |
| <i>p</i> -Coumaric acid | 0.1906 ± 0.0173 | 0.2420 ± 0.0219 | 2.018 ± 0.163 | 0.32 ± 0.031 |
| <i>t</i> -Ferulic acid | 1.0814 ± 0.0973 | 2.1751 ± 0.2131 | 0.482 ± 0.038 | 0.283 ± 0.045 |

found ineffective in presence of *p*-coumaric acid owing to higher antioxidant and reduced oxidative abrasions. This study indicates oxidative *p*-coumaric acid enriched soy based foods are healthier and can be used for biofortification of foods (Bio-fortification). Similarly, *t*-ferulic acid is another phenolic compound that reduces the lipid peroxidation in cell membrane (Del *et al.* 2013). In certain countries ferulic acid has been approved as food additive to inhibit lipid peroxidation in food stuffs. Phenolic contents of seed coat from the staple foods may protect from different types of cancer and presence of antioxidants properties from phenolic contents in food stuff lowers the risk of atherosclerosis and coronary heart disease (Xu *et al.* 2007). Hence, consumption of soybean with enriched TPC and antioxidants can provide valuable nutraceuticals that help from oxidative stress and damage. The solvent system AWAA-1 comprised of acetone-water-acetic acid in ratio of 70:28:2 resulted higher TPC. Kalitur and JS 76–205 displayed higher TPC content than other 35 soybean genotypes. The chromatogram of HPLC revealed the presence of protocatechuic acid and *p*-coumaric acid in soybean seed coats and cotyledons of 35 soybean genotypes. Presence of significant phenolic compounds in soybean implies that these genotypes can be used as nutraceuticals that have nutritional and medicinal value.

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