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
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, TAMIS-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, MAUS-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 mortal 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 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 (Złotek et al. 2016). DPPH (1 mM) solution was prepared in absolute ethanol. The seed coat and cotyledon extracts (100 µL) were 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.

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

where \(A_{\text{con}}\) O.D of blank solution; \(B_{\text{test}}\) 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 Ascensosao 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 WAa-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
compounds to brown pigments by forming $\alpha$-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 JS75-205 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
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 kidney damages induced by cisplatin. Biomedicines 28: 18.


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

<table>
<thead>
<tr>
<th>Phenolics</th>
<th>JS-76205</th>
<th>Harasoya</th>
<th>Kalitur</th>
<th>RAUS-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocatechuic acid</td>
<td>1.2623 ± 0.0931</td>
<td>0.0059 ± 0.0004</td>
<td>3.4114 ± 0.3112</td>
<td>0.0308 ± 0.0029</td>
</tr>
<tr>
<td>4-Hydroxy benzoic acid</td>
<td>0.1076 ± 0.0105</td>
<td>0.0271 ± 0.0022</td>
<td>0.1169 ± 0.0108</td>
<td>0.1059 ± 0.0098</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0.0611 ± 0.0061</td>
<td>0.4581 ± 0.0413</td>
<td>0.4343 ± 0.0425</td>
<td>0.5611 ± 0.0523</td>
</tr>
<tr>
<td>4-Hydroxy benzaldehyde</td>
<td>0.9048 ± 0.0836</td>
<td>0.9827 ± 0.0923</td>
<td>1.3953 ± 0.1014</td>
<td>1.3711 ± 0.1132</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.0275 ± 0.0021</td>
<td>0.0229 ± 0.0019</td>
<td>0.0645 ± 0.0058</td>
<td>0.0279 ± 0.0023</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.0335 ± 0.0029</td>
<td>0.0918 ± 0.0083</td>
<td>0.1276 ± 0.0112</td>
<td>0.1810 ± 0.0168</td>
</tr>
<tr>
<td>t-Ferulic acid</td>
<td>0.0977 ± 0.0093</td>
<td>0.7457 ± 0.0718</td>
<td>0.8672 ± 0.0832</td>
<td>1.3259 ± 0.1214</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Soybean cotyledon</th>
<th>JS-76205</th>
<th>Harasoya</th>
<th>Kalitur</th>
<th>RAUS-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocatechuic acid</td>
<td>0.0439 ± 0.0036</td>
<td>0.0519 ± 0.005</td>
<td>1.307 ± 0.094</td>
<td>0.0208 ± 0.0029</td>
</tr>
<tr>
<td>4-Hydroxy benzoic acid</td>
<td>0.0894 ± 0.0088</td>
<td>0.0342 ± 0.0031</td>
<td>0.202 ± 0.018</td>
<td>0.254 ± 0.022</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>0.4614 ± 0.0441</td>
<td>0.9702 ± 0.0936</td>
<td>0.638 ± 0.049</td>
<td>0.3611 ± 0.0523</td>
</tr>
<tr>
<td>4-Hydroxy benzaldehyde</td>
<td>1.2331 ± 0.1211</td>
<td>2.3264 ± 0.2121</td>
<td>0.532 ± 0.046</td>
<td>0.3111 ± 0.1132</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.0251 ± 0.0024</td>
<td>0.0332 ± 0.0031</td>
<td>0.0645 ± 0.0058</td>
<td>0.0279 ± 0.0023</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.1906 ± 0.0173</td>
<td>0.2420 ± 0.0219</td>
<td>2.018 ± 0.163</td>
<td>0.32 ± 0.031</td>
</tr>
<tr>
<td>t-Ferulic acid</td>
<td>1.0814 ± 0.0973</td>
<td>2.1751 ± 0.2131</td>
<td>0.482 ± 0.038</td>
<td>0.283 ± 0.045</td>
</tr>
</tbody>
</table>

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