Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

**E-ISSN:** 2278-4136
**P-ISSN:** 2349-8234
JPP 2019; 8(1): 1376-1378
Received: 15-11-2018
Accepted: 20-12-2018

**S Sharma**
Department of Food Science, Nutrition and Technology, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

**R Verma**
Department of Food Science, Nutrition and Technology, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

**N Singh**
Department of Food Science, Nutrition and Technology, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

**YS Dhaliwal**
Department of Food Science, Nutrition and Technology, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

**Correspondence**
**S Sharma**
Department of Food Science, Nutrition and Technology, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India

# Assessment of anti nutritional factors and antioxidants in three genotypes of adzuki beans

**S Sharma, R Verma, N Singh and YS Dhaliwal**

**Abstract**
The basic purpose of the study was to assess and compare the anti nutritional factors and antioxidant activity of the three genotypes of the adzuki bean viz. *Local Totru*, HPU-51 and EC-340264. Anti nutritional factors like phytic acid and trypsin inhibitors were assessed along with the flavonoid content of the adzuki bean seeds. The phytic acid content of the seeds ranged from 368.96 to 507.92 mg/100g. The percent inhibition of the seeds ranged from the 52 to 77 percent in all the three genotypes. The study can be useful for the producer and consumer to harvest the benefits of adzuki bean based value added products.

**Keywords:** adzuki bean, trypsin inhibitors, phytic acid, antioxidant activity

## Introduction
Adzuki bean [*Vigna angularis* (Willd.) Ohwi and Ohashi], syn. [*Phaseolus angularis* (Willd.)], (*Dolichos angularis* Willd.) and [*Azukia angularis* (Willd.) Ohwi] of Leguminosae family is an erect, bushy annual, usually 30-40 cm tall, determinate, slightly viny with trifoliate leaves and short, auxillary inflorescence having 6-12 clustered bright yellow flowers. Pods are cylindrical, 6-12 cm long with 4 – 12 seeds and straw coloured with blackish or brown forms and constricted between seeds. It is a self-pollinated crop and the seeds are cylindrical to cordate with smooth, wine red, occasionally buff, black or mottled seed coat. It is a traditional pulse crop in East Asia and widely used as a source of protein for human nutrition, especially in developing countries. It mainly occurs in temperate and sub temperate regions (Rubatzky and Yamaguchi, 1997) [6].
In India, its cultivation is confined to North-eastern and Northern hill zones. It is sporadically grown in Kangra, Chamba, Mandi and Sirmour districts of Himachal Pradesh (Shweta, 2013) [8]. Adzuki bean grows in all types of soil and is more tolerant to heavy rainfall than other grain legumes.
Also known as small red beans, they are a popular ingredient in many confections in the orient. The predominant use of adzuki bean in traditional Japanese confections is a paste or *wagashi* such as *youkan, manju* and *amanatto.* Adzuki bean is a rich source of carbohydrates, protein, vitamins, minerals and fiber (Tjahjadi *et al*., 1988) [10], however they also contain antinutritional factors. Phytates, α- galactosides and trypsin inhibitors are among these factors, and their concentrations differ widely among the different cultivars of adzuki beans.
The bioactive compounds in the adzuki bean seed coat have received significant interest because of their health-promoting antioxidant properties. High levels of phenolic compounds, including flavonoids in the seed are attributed to their strongly coloured maroon seed coats, these mainly contain proanthocyanins, which have extremely high *in vitro* radical scavenging activity (Lin and Lai, 2006) [3].
The objective of the present study is to assess the three genotypes of adzuki bean viz. viz. *Local Totru*, HPU-51 and EC-340264 for their antioxidant activity using DPPH scavenging activity and its anti nutritional factors like phytic acid and trypsin inhibitor activity.

## Material and Method
### Procurement of raw material and sample preparation
Different genotypes of adzuki bean were procured from the Department of Organic Agriculture, College of Agriculture, CSK Himachal Pradesh Agricultural University, Palampur. Chemicals and other ingredients required for analysis and product development were procured from the reputed local suppliers.
Initially procured adzuki beans samples were cleaned to remove damaged seeds, dust and other foreign materials. For analyzing different parameters the adzuki beans were ground into fine flour.

**Anti nutritional factor**

**Phytic acid:** Phytic acid was determined by the method Haugh and Lantzch (1983) [2]. One gram of finely ground sample was extracted with 25 ml of hydrochloride acid for three hours with continuous shaking in a shaker. After proper shaking, it was filtered through Whatman no 1 filter paper and volume was made 25 ml with 0.2 N hydrochloride acid. An aliquot of 0.5 ml was pipetted of the above extract into test tubes fitted with a glass stopper. Added 1 ml of ferric ammonium sulphate solution. Heated the tube and centrifuged for 30 min at 3000 rpm. Transferred one ml supernatant to another test tube and added 1.5 ml bi-pyridine solution. Measured the absorbance at 519 nm against distilled water. For plotting a standard curve different concentrations i.e. 0.2 to 1.0 ml of standard sodium phytate solution containing 40 – 200 µg phytic acid were taken and made to 1.4 ml with water. The optical density of 0.500 corresponded to 120 µg phytic acid.

**Calculations**

$$\text{Phytic acid (mg/100g)} = \frac{\text{Reading of graph x ml of volume made}}{\text{Weight of sample x aliquot taken}} \text{ X } 100$$

**Trypsin inhibitor**

The trypsin inhibitor activity of extract (sample + saline solution) was determined by determining its ability to inhibit caseinolytic activity of trypsin using the method of Ray and Rao, (1971) [5]. In one gram of ground sample about ten ml of 1 per cent NaCl was added and shaken for three hours and kept overnight in refrigerator and centrifuged. The supernatant was collected and the sample was re-extracted with NaCl. The final volume was adjusted to 25 ml with saline. Different sets of incubates prepared. These incubates were centrifuged for 10 minutes. Estimation of total proteins was done by the method of Lowry *et al.* (1951).

**Trypsin inhibitor unit**

One unit of trypsin has been defined as amount of enzyme which converts 1 mg of casein to TCA soluble components at 37ºC in 30 min at pH 7.0 and 1 unit of inhibitor activity of trypsin by one unit under the assay conditions.

**Calculations**

1ml contains 10 mg casein

$$\text{TIU/g} = \frac{\text{O.D of test} \times \text{conc of std} \times \text{dilution factor} \times 100 \times 1/10}{\text{O.D of std} \times \text{wt of sample}}$$

**DPPH radical scavenging activity**

To a known volume of 20,40,60,80,100 µl of 1mM of L-Ascorbic acid taken in five sets test tubes and then added methanol to make the volume of 3 ml DPPH solution 200 µM, 1ml prepared in methanol was added to the test tubes containing methanolic solutions of ascorbic acid. The contents were vortex properly and allowed to incubate at 30ºC for 30 minutes in the dark. Absorbance was measured at 517nm of the spectrophotometer. The per cent of DPPH free radical scavenging activity (% inhibition) was calculated using following equation.

$$\% \text{ Inhibition} = \frac{\text{Abs (control)} - \text{Abs (test)}}{\text{Abs (control)}} \times 100$$

$IC_{50}$ value (the amount of antioxidant necessary to decrease the initial DPPH free radical concentration by 50 percent) was calculated from the regression line obtained from the plot of per cent inhibition against concentration of solution using the following equation

$$IC_{50} = \frac{\text{(50-y intercept)}}{\text{Slope}}$$

**Flavonoids**

Flavonoids were assessed using the method of Boham and Kocipia, (1994) [1]. Ten gram of sample was extracted repeatedly with 100 ml of 80 per cent aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no 42 (125 mm). The filterate was later transferred in to a crucible and evaporated to dryness over water bath and weighed. The flavonoid content was calculated in per cent.

$$\text{Flavonoid (\%)} = \frac{\text{Weight of the crucible with sample} - \text{weight of the empty crucible}}{\text{Weight of the sample}} \times 100$$

**Result and discussion**

Bean seeds contain a number of anti-nutritional compounds which can be of proteinous or non proteinous nature. The most characterized protein inhibitors of legumes seeds are trypsin inhibitor. The presence of protease inhibitor in food decreases the apparent nutritional quality of proteins in the diet by affecting the ability of body digestive enzymes to degrade dietary protein thus limiting the intake of amino acids needed to construct new protein. However, in certain situation the effects of the inhibitors on protein digestion might be advantageous e.g. by improving the intact absorption of some therapeutic proteins such as orally delivered insulin. Phytic acid is chelator of important minerals as calcium, magnesium, iron and zinc etc. It interferes with their absorption and utilization and thereby contributes to mineral deficiency (Vasagam and Rajkumar, 2011) [11].

Antinutritional factors in adzuki bean are listed in the Table 1. Trypsin inhibitor units were found in the range of 2881.12 to 3510.07 (TIU/g), with the highest content of trypsin inhibitor detected in Local *Totru* (3510.07 TIU/g) and lowest in HPU-51 (2881.12 TIU/g). Sai *et al.* (2009) [9] reported 509.53 g/100g of trypsin inhibitor in adzuki bean. A study conducted on the three legume seeds of royal project foundation (navy bean, red kidney bean and adzuki bean) by Wati *et al.* (2009) [12] concluded the trypsin inhibitor content in the range of 8490 to 12354 TIU/g with different extraction medium in adzuki bean. However, in present study the trypsin inhibitor is found to be less than the reported values, which could be attributed to the varietal differences, environmental conditions and differences in the medium of extraction.

**Table 1:** Anti-nutritional factors of different genotypes of adzuki bean seeds

| S No. | Parameters | Genotypes | | | CD (P≤0.05) |
|---|---|---|---|---|---|
| | | EC-340264 | HPU-51 | Local *Totru* | |
| 1. | Trypsin inhibitor (TIU/g) | 3146.85 | 2881.12 | 3510.07 | 115.4 |
| 2. | Phytic acid (mg/100g) | 507.92 | 368.96 | 429.67 | 33.6 |

The phytic acid content was observed in the range of 368.96 to 507.92 mg/100g. In all the three genotypes the highest phytic acid content was observed in EC-340264 (507.92mg/100g) and the lowest value in HPU-51 (368.96mg/100g). The results are in agreement with those reported by Shweta, (2013) [8], who reported 563 mg/100g of phytic acid content in the adzuki bean seeds. The results revealed that the HPU -51 genotype of adzuki bean contain lesser amount of phytic acid and trypsin inhibitors in comparison to the other two genotypes.

**Anti-oxidant properties of adzuki bean**

The flavonoid content in the adzuki bean seeds ranged from 5.10 to 6.80 per cent given in Table 2. The highest flavonoid per cent was detected in HPU-51(6.80 %) and lowest in Local *Totru* (5.10 %) per cent. The per cent inhibition of the adzuki bean revealed that Local *Totru* genotype had highest i.e. 77.00 per cent inhibition followed by HPU-51 (62.00%) and lowest in the EC-340264 (52.00%). The lower the value of $IC_{50}$ the higher will be the antioxidant activity (Sanchez and Moreno et. al. 1999) [7]. As per this, Local *Totru* (77.00%) was found to have lowest antioxidant activity while EC-340264 (52.00%) had the highest amount of antioxidant activity.

**Table 2:** Antioxidant properties of different genotypes of adzuki bean

| S. No. | Parameters | Genotypes | | | CD (P≤0.05) |
|---|---|---|---|---|---|
| | | EC-340264 | HPU-51 | Local *Totru* | |
| 1. | Flavonoid (%) | 5.23 | 6.80 | 5.10 | 2.21 |
| 2. | $IC_{50}$ (% inhibition) | 52.00 | 62.00 | 77.00 | NS |

**Conclusion**

Adzuki bean is one of the most important food crops which has different pigments that are known to include polyphenols such as proanthocyanidins and quercitin, which have therefore received considerable attention owing to their well documented antioxidant activities. However it also have a considerable amount of phytic acid and trypsin inhibitor content, the study revealed that HPU-51 has lesser phytic acid and trypsin inhibitor activity while a higher DPPH scavenging activity was observed in EC -340264 in comparison to the *Local Totru* and HPU -51 genotypes of adzuki bean.

**References**


1. Boham AB, Kocipia AC. Flavonoid and condensed tannins from leaves of Hawaiian vaccinimum vaticulum and vicalycinium. Pacific Sciences. 1994; 48:458-463.
2. Haugh W, Lantzch HJ. Sensitive method for the rapid determination of phytates in cereals and cereal products. Journal of the Science of Food and Agriculture. 1983; 34:1423-1427.
3. Lin PY, Lai HM. Bioactive compounds in legumes and their germinated products. Journal of Agricultural and Food Chemistry. 2006; 54:3807-3814.
4. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Science. 2001; 8:203-208.
5. Ray DN, Rao PS. Evidence of isolation purification and some properties of trypsin inhibitors activity in *Lathyrus sativus*. Journal of Agricultural and Food Chemistry 1971; 19:257.
6. Rubatzky VE, Yamaguchi M. World vegetables: Principles, production, and nutritive values. 2nd ed. Chapman & Hall, New York, 1997, 572.
7. Sanchez VR, Clemente A, Vioque J, Bautista J, Millan F. Protein isolates from chickpea (*Cicer arietinum* L.): Chemical composition, functional properties and protein characterization. Food Chemistry. 1999; 64:237-243.
8. Shweta. Study on the Biochemical profile of different VIGNA SPP. prevalent under mid-hill conditions of Himachal Pradesh. PhD Thesis, Department of Chemistry and Biochemistry, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India, 2013, 4-66.
9. Sai SS, Ketnawa S, Chaiwut P, Rawdkuen. Biochemical and functional properties of proteins from red kidney, navy and adzuki beans. Asian Journal of Food and Agro Industry. 2009; 2:493-504.
10. Tjahjadi C, Lin S, Breene WM. Isolation and characterization of adzuki bean (*Vigna angularis* cv Takara) Proteins. Journal of Food Science. 1988; 53:1438-1443.
11. Vasagam KPK, Rajkumar M. Beneficial influences of germination and subsequent autoclaving of grain legumes on proximate composition, antinutritional factors and apparent digestibility in black tiger shrimp, *Penaeus monodon Fabricius*. Aquaculture Nutrition. 2011; 17:188-195.
12. Wati RK, Theppakorn T, Rawdkeun. Extraction of trypsin inhibitor from three legume seeds of the royal project foundation. Asian Journal of Food and Agro Industry. 2009; 2:245-254.