Research Article

Variability in Polyphenols, Antioxidants and Mineral Composition in Different Genotypes of Pigeonpea (*Cajanus Cajan*) Grown in India

Chetna Chugh[1] and Ritu[2]

[1]Indian Agriculture Research Institute, New Delhi
[2]Maharshi Dayanand University, Rohtak

Abstract

The study was conducted to evaluate the polyphenols, antioxidants and minerals composition in different genotypes of pigeonpea [*Cajanus cajan* (L.) Millsp]. Different assays were performed to measure total phenolic content, total flavonoid and antioxidant activities were measured using 2,2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Ferric reducing antioxidant power assay (FRAP) and mineral composition was measured using Atomic absorption spectrophotometer. The total phenolic content ranged from 5.1 to 290.2 mg GAE/g dry weight. Pusa 992 had maximum amount of phenolics in 80% acetone extract. The total flavonoid content (TFC) varied from 0.17 to 3.62mg QE/g with IPA 203 having highest TFC value in 100% methanol. DPPH activity varied from 0.18mM in Manak to 0.87 mM in IPA 203 TE per g dry seed weight. FRAP varied from 0.62mM in LGR-38 to 10.15 mM in UPAS 120. Diverse genotypes were evaluated in relation to the content of 6 minerals (Zn, Cu, Fe, Ca, Mg, and Na) important for human nutrition. The level of Ca, Mg, Na, Zn, Cu, Fe ranged from 1-19.8, 12-124.6, 2.8-6.9, 2.3-3.7, 0.20-.097, 4.3-24.1 mg/100g respectively among different genotypes of pigeonpea. The information of this study will increase the understanding of the function of the pigeonpea in the diet to reduce chronic diseases and also be used for selecting superior genotypes for breeding programmes.

Keywords: Antioxidants, 2,2- diphenyl-1-picrylhydrazyl, Polyphenols, Total flavonoid content, Total phenolic content

***Correspondence**
Author: Chetna Chugh
Email: chetnachugh@gmail.com

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp] have diverse biological activities such as antioxidant, anti-aging, anti-cancer, anti-inflammation, anti-atherosclerosis, cardiovascular protection, improvement in endothelial function, as well as inhibition of angiogenesis and cell proliferation activity for disease prevention and health promotion [1-3]. It is widely accepted that significant antioxidant activity of food is related to high total phenolic content. It contains a large variety of phenolic derivatives, including simple phenols, phytosterols, saponins, phenyl propanoids, benzoic acid derivatives, flavonoids, stilbenes, tannins, lignans and lignins [4]. Natural polyphenols exert their beneûcial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases. Numerous studies have reported the bioactive component such as vanillic acid, caffeic acid, cajaninstilbene acid, pinostrobin and vitexin from different parts such as leaves, roots, stem and seeds which have biological and pharmacological activities [1-3]. Pigeonpea seeds are used for treating kidney ailments, measles, hepatitis and sickle cell anemia [5-7]. Pigeonpea seeds are consumed as a grain crop and also used as a fodder for animals. This fodder is a rich source of fibers and protein. In the perspective of nutritional and medicinal attributes of *Cajanus cajan*, characterization and compositional analysis of pigeonpea seeds are of great importance. The phytochemical analysis depends both on genetic and environment. The present investigation was undertaken to develop on efficient protocol for determining total phenolic content, antioxidants and mineral composition in seeds of pigeonpea.

Materials and Methods

Different cultivars of pigeonpea such as Pusa 33, Pusa 992, Pusa 855, Pusa 84, Pusa 2001, Pusa 2002, LGR 38, Narendar Arhar-1, UPAS 120, IPA 203, Manak, Paras were procured from Pulses Research Laboratory Pusa, New Delhi, Indian Institute of Pulses Research, Kanpur and National Seed Corporation, Rohtak respectively. They were stored at 4°C until use. Legume seed flour was extracted with three different solvents ethanol (70-80%), methanol (70-100%) and acetone (80%). Different assays were performed to measure total phenolic content, total flavonoid and

antioxidant activities were measured using 2,2- diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging, Ferric reducing antioxidant power assay [8] All extractions were conducted in triplicates.

The extracts were analyzed for total phenolic content using the Folin- Ciocalteu method [9, 10]. Gallic acid was used as the reference standard. Total phenol content was expressed as mg of Gallic acid equivalents (GAE)/g of sample.

For flavonoid estimation aluminum chloride colorimetric method was used [11]. Each plant extract were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV visible spectrophotometer. The calibration curve of quercetin solutions was prepared at concentrations 0.0062 to 0.18 mg/ml in 70% Ethanol, 100% Methanol and 80% Acetone separately. TFC was expressed as mg quercetin equivalents/ g of dry sample.

Ferric reducing antioxidant power (FRAP) method was used for determination of antioxidant activity of the sample [12]. To 150 µL extract 4.5 mL FRAP reagent was added. The absorbance readings were started after 5 min and they were performed at 593 nm. The blank consisted of FRAP reagent. The final absorbance of each sample was compared with those obtained from the standard curve made from ferric sulfate heptahydrate (1-5 mM/L). Results were expressed as antioxidant concentration in mM.

DPPH-free radical scavenging capacity of pigeonpea extracts was evaluated according to the method of [13] with slightly modifications. Briefly, a dose of 0.2 mL of the pigeonpea extract was added to 3.8 mL ethanol and methanol solution of DPPH radical (final concentration was 0.1 mM). The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance for the samples was measured using the UV 160 spectrophotometer at 517 nm against ethanol and methanol blank respectively. The free radical scavenging activity of pigeonpea extracts was expressed as an equivalent of that of Trolox. Every sample was extracted in triplicate, and the results were calculated and expressed as micromoles of Trolox equivalents per gram of legume using the calibration curve of Trolox with linearity range 0.8-4 mM. All the experiments were performed in triplicates, and the results were expressed as mean ± SD (standard deviation) statistical analysis was performed using excel 2007.

Results and Discussion

Total phenolic content

The present study was undertaken to compare the phytochemical profiles, precisely the total phenolic content, total flavonoid content and antioxidant activity of seed extracts of diverse Indian genotypes of pigeonpea and the effect of different organic solvents used for extraction. The total phenolic content and antioxidant activity is influenced by the type of polarity of solvents, amount of solvent used and the genotype of pigeonpea seeds phenolic compounds are a class of antioxidant agents which act as free radical terminators. Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al*. 2003). Phenolic compounds contribute to the overall antioxidant activities of the plant foods. Total phenolic content was estimated using FC method. Three different solvents have been employed for the extraction purpose of 12 cultivars of pigeonpea. The TPC value of twelve different cultivars of pigeonpea varies significantly ($P < 0.05$) with the genotypes and the type of solvent used (**Table 1**). The TPC yields in terms of extraction solvents were in the following order from high to low 80% Acetone> 70% Ethanol> 100% Methanol for Pusa 33, Pusa 84, Pusa 992, UPAS 120, NDA, Pusa 2001, Pusa 2002 and 80% Acetone> 100% Methanol> 70% Ethanol for LGR 38, IPA 203, Manak and Paras. The results suggest that 80% Acetone gave the highest yield among three solvents for extracting total phenolics of pigeonpea. The mean TPC of the pigeonpea crude extract of all the selected cultivars was higher than that reported for pigeonpea crude water extract of *C. cajan* brown cultivar in Nigeria (1.2±0.2 mg/g) [14]. Differences may be due to the genotype of pigeonpea and different extraction methods.

Total flavonoid content

Flavonoids are plant secondary metabolites, including flavones, flavanols, and condensed tannins. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

TFC of the extracts were analyzed and the results are presented in **Table 2**. The TFC yields in terms of extraction solvents were in the following order from high to low 100% Methanol> 80% Acetone> 70% Ethanol for Pusa 33, Pusa 84, Pusa 2001, Pusa 2002, UPAS 120, IPA 203, NDA, LGR 38, Manak and Paras; 80% Acetone> 100% Methanol> 70% Ethanol for Pusa 992, and Pusa 855. The results suggest that 100% Methanol gave the highest yield

for above mentioned cultivars; 70% Ethanol gave the lowest yield in all the cultivars. The mean TFC of the pigeonpea crude extract of all the selected cultivars was higher than chickpea crude extract in different solvents (0.18 to 3.16 mg CE/g), lentil (0.72 to 2.21 mg CE/g) and yellow soybean (0.25 to 0.41 mg CE/g) (Wu *et al.* 2009). However, all the cultivars possessed lower flavonoid content than the crude ethanol extract (293.45±3.12 mg/g) extract of leaves of pigeonpea [8].

Table 1 Total phenol content (mg GAE/g) of pigeonpea genotypes as affected by different solvents

Variety	100% Methanol	70% Ethanol	80% Acetone
LGR 38	5.1 ± 0.45^{a}	4.7 ± 0.78^{a}	96.4 ± 0.47^{a}
Pusa 33	14.48 ± 0.23^{b}	23.99 ± 0.45^{d}	247.7
Pusa 84	17.7 ± 0.92^{c}	62.8 ± 3.9^{e}	257.2 ± 5.9^{c}
Pusa 992	26.8 ± 0.61^{d}	41.8 ± 0.51^{f}	290.2 ± 3.3^{d}
UPAS 120	48.6 ± 0.80^{e}	55.6 ± 1.3^{g}	75 ± 0.80^{e}
NDA 1	15 ± 1.00^{bf}	25.7 ± 1.5^{g}	91.4 ± 1.7^{f}
IPA 203	10.1 ± 0.36^{g}	9.6 ± 1.6^{h}	38 ± 1.5^{g}
Pusa 855	17.5 ± 1.00^{cfh}	39 ± 2.4^{h}	217.1 ± 2.4^{h}
Pusa 2001	14 ± 0.15^{i}	8.9 ± 0.11^{a}	154.14 ± 0.11^{i}
Pusa 2002	$15.58 \pm 1.3b^{cfhj}$	19.5 ± 0.20^{b}	203 ± 0.17^{j}
Manak	12.6 ± 0.15^{k}	11.8 ± 0.09^{c}	127.3 ± 0.43^{k}
Paras	14.1 ± 0.47^{bfl}	13.2 ± 0.88^{c}	185.4 ± 0.17^{l}

Data are expressed as mean ± standard deviation (n = 3); values with in each type of variety marked by same letter with in same column are not significantly different ($P < 0.05$).

Table 2 Total flavonoid contents (mg QE/g) of different pigeonpea genotypes using different solvents

Variety	100% Methanol	70% Ethanol	80% Acetone
LGR 38	1.40 ± 0.03^{a}	0.17 ± 0.01^{a}	1.38 ± 0.06^{a}
Pusa 33	2.56 ± 0.04^{b}	0.93 ± 0.08^{b}	2.11 ± 0.17^{b}
Pusa 84	2.58 ± 0.14^{bc}	0.17 ± 0.006^{ac}	1.33 ± 0.12^{ac}
Pusa 992	1.63 ± 0.14^{de}	0.46 ± 0.006^{d}	2.08 ± 0.13^{bd}
UPAS 120	3.50 ± 0.09^{e}	0.88 ± 0.01^{be}	2.01 ± 0.17^{be}
NDA 1	3.23 ± 0.17^{e}	0.56 ± 0.07^{df}	1.81 ± 0.04^{e}
IPA 203	3.62 ± 0.09^{ef}	0.22 ± 0.03^{ag}	1.74 ± 0.08^{ef}
Pusa 855	3.21 ± 0.11^{eg}	0.26 ± 0.01^{g}	1.58 ± 0.05^{g}
Pusa 2001	2.50 ± 0.06^{bch}	0.83 ± 0.02^{bh}	2.10 ± 0.18^{bdh}
Pusa 2002	2.78 ± 0.06^{ci}	0.59 ± 002^{fi}	1.95 ± 0.06^{bh}
Manak	$1.75 \pm .04d^{j}$	0.86 ± 0.01^{behj}	2.44 ± 0.19^{bi}
Paras	3.48 ± 0.09^{ek}	0.61 ± 0.01^{fk}	2.15 ± 0.03^{bhj}

Data are expressed s mean ± standard deviation (n = 3); values with in each type of variety marked by same letter with in same column are not significant.

Ferric reducing antioxidant power

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of [15]. At low pH, reduction of ferric tripyridyl triazine (Fe TPTZ) complex to ferrous form (which has an intense blue color) can be monitored by measuring the change in the absorption at 530 nm. The change in absorbance is therefore, directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture. The FRAP values of the antioxidant extracts from selected pigeonpea cultivars are presented in **Table 3**. Extracts differed significantly ($P < 0.05$) in their antioxidant activity with the genotype and type of extraction solvent used. Results showed that UPAS 120 (10.15 ± 0.00) possessed the highest antioxidant activity. LGR-38 (0.62 ± 0.05) has least antioxidant activity Mean FRAP values of crude extracts of selected pigeonpea cultivars was higher than crude extract solutions of *Vigna sinensis* in chloroform, ethyl acetate and methanol (0.022 to 0.143 mM) (Xu and Chang 2007).

DPPH assay

The principle of DPPH radical scavenging assay is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Due to the presence of an odd electron it gives a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased. The antioxidant capacity based on the DPPH free radical scavenging ability of the seed extract was expressed as mmol Trolox equivalents (TEAC) per gram of extract. Extracts from different extraction solvent differed significantly (P< 0.005) in their TEAC value in six pigeonpea genotypes (**Table 4**).

Table 3 FRAP values of different pigeonpea genotypes as affected by different solvents

Variety	100% Methanol	70% Ethanol	80% Acetone
LGR 38	0.62 ± 0.05^{a}	0.72 ± 0.02^{a}	6.17 ± 0.00^{a}
Pusa 33	1.02 ± 0.01^{bc}	1.41 ± 0.01^{bc}	7.04 ± 0.01^{bc}
Pusa 84	1.99 ± 0.03^{cd}	0.66 ± 0.01^{cd}	2.02 ± 0.02^{cd}
Pusa 992	1.75 ± 0.03^{de}	1.38 ± 0.05^{bcd}	9.7 ± 0.00^{de}
UPAS 120	2.36 ± 0.09^{ef}	5.1 ± 0.06^{ef}	10.15 ± 0.00^{ef}
NDA 1	1.56 ± 0.01^{fg}	1.04 ± 0.01^{fg}	8.87 ± 0.15^{fg}
IPA 203	1.17 ± 0.05^{gh}	1.31 ± 0.03^{gh}	3.63 ± 0.06^{gh}
Pusa 855	1.5 ± 0.02^{hi}	2.11 ± 0.05^{hi}	4.5 ± 0.07^{hi}
Pusa 2001	1.43 ± 0.06^{ij}	1.7 ± 0.13^{ij}	6.68 ± 0.00^{ij}
Pusa 2002	1.07 ± 0.07^{bcj}	1.45 ± 0.13^{bcj}	7.23 ± 0.20^{bcj}
Manak	0.79 ± 0.08^{kl}	1.13 ± 0.03^{kl}	6.57 ± 0.02^{kl}
Paras	0.64 ± 0.01^{lm}	1.06 ± 0.07^{lm}	6.42 ± 0.03^{lm}

Data are expressed s mean ± standard deviation (n = 3); values with in each type of variety marked by same letter with in same column are not significantly different (P < 0.05).

Analysis of mineral profile

Six different cultivars with better antioxidant activity were tested for mineral content as shown in **Table 5**. Seed material was digested and the sample solution was analyzed for the content of six different minerals, viz. Ca, Mg, Na, Zn, Cu and Fe by Atomic Absorption Spectrophotometer. Table 5 shows the minerals analyzed in four different pigeonpea seed cultivars. The level of Ca, Mg, Na, Zn, Cu, Fe ranged from 1-19.8; 12-124.6; 2.8-6.9; 2.3-3.7; 0.20-.097; 4.3-24.1 mg/100g respectively among four different pigeonpea cultivars.

Table 4 DPPH values of pigeonpea genotypes as affected by different solvents

Variety	100% Methanol	70% Ethanol
Pusa 992	0.246 ± 0.006	041 ± 0.005^{a}
Pusa 2002	0.427 ± 0.01^{bc}	0.73 ± 0.04^{bc}
NDA	0.81 ± 0.08^{cd}	0.99 ± 0.006^{cd}
IPA	0.879 ± 0.010^{de}	1.03 ± 0.014^{bcd}
Pusa 84	0.373 ± 0.017^{ef}	0.82 ± 0.010^{ef}
Manak	0.184 ± 0.004^{fg}	0.249 ± 0.022^{fg}

Data are expressed s mean ± standard deviation (n = 3); values with in each type of variety marked by same letter with in same column are not significantly different (P < 0.05).

Table 5 Mineral contents (mg/100g seed extract) in different pigeonpea genotypes

Minerals	UPAS 120	Pusa 2001	Pusa 33	IPA 203	Manak	Pusa 2002
Ca	19.8	14.3	19.4	1.9	1.4	1
Mg	124.2	111.9	124.6	12.17	104.5	112.2
Fe	24.1	7.1	4.3	9	6	4.4
Na	6.9	2.8	2.9	2.8	3.9	3.8
Zn	3.7	3.1	2.9	3	3	2.3
Cu	0.97	0.62	0.58	0.28	0.54	0.20

Nutritional evaluation of different pigeonpea is generally very essential. Minerals were found to play very important role in human health [16] reported a significant decrease of systolic blood pressure with calcium supplementation for the hypertensive persons, since magnesium works in conjunction with calcium to help in transmitting nerve impulse to the brain [17]. Several studies showed that iron as a component of hemoglobin in blood is needed for oxygen transport. Similarly several other minerals also have important role in human health.

Conclusion

This study indicated that pigeonpea, used widely for human consumption exhibited significant ferric reducing power, total phenolic as well as flavonoid content. These factors conclude that pigeonpea is a leguminous species that can provide an important daily source of phenolic compounds in human diet. Therefore this research could be useful to evaluate the desirable trait in pigeonpea by breeding programmes for the selection of cultivars with high nutritive value and for the improvement of seed nutrition quality traits.The genotypes with higher antioxidant activity may be assessed for abiotic stress tolerance and thus used as superior breeding material

References

[1] Wu N, Fu K, Fu YJ, Zu Y G, Chang F R and Chen Y H. 2009. Antioxidant activities of extracts and main components of pigeonpea leaves. Molecules 14: 1 032–43.

[2] Luo M, Liu X, Zu Y, Fu Y, Zhang S and Yao L. 2010. Cajanol, a novel anticancer agent from pigeonpea [Cajanus cajan (L.) Millsp.] roots, induces apoptosis in human breast cancer cells through a ROS mediated mitochondrial pathway. Chem Biol Interac 188: 151–60.

[3] Kong Y, Fu Y J, Zu Y G, Chang F R, Chen Y H and Liu X L. 2010. Cajanus lactone a new coumarin with anti bacterial activity from pigeonpea leaves. Food Chemistry 121: 1 150–5.

[4] Shahidi F and Naczk M. 2004. Antioxidant properties of food phenolics. (In) Phenolics in Food and Nutraceuticals, p 1 403. Shahidi F, Naczk M (Eds). CRC Press, Boca Raton, Fla.

[5] Abbiw D K. 1990. Useful Plants of Ghana. Richmond Intermediate Technology Publications and Royal Botanic Gardens, Kew, London, UK.

[6] Amalraj T and Ignacimuthu S. 1998. Evaluation of the hypoglycemic effect of Cajanus cajan (seeds) in mice. Indian Journal of Experimental Biology 36: 1 032–3.

[7] Grover J K, Yadav S and Vats V J. 2002. Medicinal plants of India with antidiabetic potential. Journal of Ethnopharmacolgy 81: 81–100.

[8] Xu and Chang A. 2007. Comparative study on phenolics profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science and Technology 72(2): 159– 67.

[9] Singleton V L and Lamuela-Raventos R M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent methods. Enzymology 299: 152–78.

[10] Singleton V L and Rossi J A. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enol Viticulture 16: 144–58.

[11] Chang C, Yang M, Wen H and Chern J. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food Drug Analysis 10: 178–82

[12] Djordjevic T M and Slavica S. 2012. Total antioxidant power in Lentil International Journal of Food Properties 46: 125–7.

[13] Chen, D H, Li H Y and Lin H. 1985. Studies on chemical constituents in pigeonpea leaves. Chinese Tra-traditional Herbal Drugs 16: 134–6.

[14] Oboh G. 2007. Antioxidant properties of some commonly consumed and underutilized tropical legumes. European Food Research and Technology 224(1): 61–5.

[15] Benzie I F F and Strain J J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP Assay. Analytical Biochemistry 239: 70–7.

[16] Emynur Shafekh S, Mohd Adzim Khalili R, Catherine C C W, Siti Syakiroh Z A, Ummu Habibah A, Norhayati A H, Nor Farhanah M Y, Noor Husna Z, Siti Nafizah M B, Azlina M, Noor Shahhida Sazura A R and Ahmad Zubaidi A L. 2012. Total phenolic content and in vitro antioxidant activity of Vigna sinensis. International Food Research Journal 19(4): 1 393– 400

[17] Hallfrisch J, Veillon C, Patterson K Y, Hill A D, Benn I, Holiday B, Burns R, Zhonnie S, Price F and Sorenson A. 2000. Toxicology 149: 143–8.

Publication History
Received 18th Jan 2018
Revised 05th Feb 2018
Accepted 15th Feb 2018
Online 28th Feb 2018