Antioxidant activity, nutraceutical profile and health relevant functionality of nine newly developed chickpea cultivars (Cicer arietinum L.)

Sharma Shruti a, Yadav Neelam a, Singh Alka b and Kumar Rajendra b
aCentre of Food Technology, Faculty of Science, University of Allahabad, Allahabad. 211002, India
bDepartment of Genetics, Indian Agriculture Research Institute (IARI), New Delhi, India
Corresponding author’s address: Dr. Neelam Yadav
Centre of Food Technology, Faculty of Science, University of Allahabad, Allahabad. U.P. Pin. 211002.
E mail: neelam_aidu@yahoo.com, Tel: +91-09415495675, Fax - 0532-2640192
Received 22 May 2013; Accepted 12 June 2013

Abstract
The present study was designed to analyze the phenolic content, antioxidant and anti-diabetic properties of newly developed desi and kabuli chickpea cultivars. Total polyphenolic content ranged from 203 to 255 (mg/100g) for desi and kabuli chickpea cultivars, respectively. Desi cultivars showed significantly higher level of nutraceutical properties than kabuli cultivars. All the investigated samples showed promising level of DPPH (67% to 88%), FRAP (23 to 35 μmolAAE/g), metal chelating activity (27% to 67%) and α-amylase inhibiting activity (49% to 62%). It is therefore concluded that all the cultivars of chickpea particularly desi cultivars can be used in the prevention of many degenerative diseases.

Keywords: Cicer arietinum L., Total polyphenol content, Flavonoid content, Antiradical activity, Metal chelating activity, Antidiabetic property.

1) Introduction
Chickpea (Cicer arietinum L.) is a premier pulse crop of Indian subcontinent. It is the world’s third most important grain legume after beans and peas [1]. Most of the world’s chickpea production and consumption (>70%) is in India. India is the largest chickpea producer as well as consumer in the world. India grows chickpea on about 6.67 million ha area producing 5.3 million tonnes which represents 30 and 38% of the national pulse acreage and production respectively [2]. This crop is of importance in many other countries also, it is grown in 37 countries in South and West Asia, North and East Africa, Southern Europe, North and South America. In Mediterranean countries it is cultivated principally as a legume crop, since it is well adapted to semi arid condition [3].

In India it is mostly consumed as cooked whole seeds. Chickpeas are valuable source of calories, protein, minerals, fibers and minor component of potential health benefits [4]. It occupies a very important place in human nutrition. Apart from other beneficial nutrients, some recent studies have pointed out that it also contains several others ‘bioactive compounds’ such as polyphenol, lectins, oligosaccharides and are categorized as ‘secondary metabolites’ of plants. These secondary metabolites are considered as anti nutrients, simultaneously conferring health benefits [5]. These bioactive compounds exert their beneficial health effects by their antioxidant activity [6]. These compounds have been found to remove free radicals, chelates metal catalysts, activate antioxidative enzymes, reduce alpha tocopherol radicals and inhibit oxidases [7]. The phenolic compounds constitute one of the most numerous and ubiquitously distributed groups of plant secondary metabolites ranging from simple molecules (e.g. phenolic acids, phenyl propanoids and falvonoids) to highly polymerized compounds (e.g. lignin and melanin). Polyphenolic compounds not only effectively prevent the oxidation of foods but they also act as a protective factor against oxidative damage in the human body [8, 9]. They may prevent the development of many diseases such as atherosclerosis, cancer, diabetes etc. As consequences of these activities, the presence of phenolic compounds in the foods has come to be viewed as beneficial element by many
scientists in recent years. Their findings stated that the antioxidant activities of these bioactive compounds are related to their chemical structure. Polyphenols are also used as a source of potentially beneficial phytochemical in the food and pharmaceutical industries. Recently, natural antioxidants which can inhibit some key enzymes, like alpha-amylase and alpha-glucosidase etc. linked to post prandial hyperglycemia have attracted lot of interest as a potential approach for curing type 2 diabetes mellitus [10, 11]. While a lot of information abounds on proximate composition, minerals and anti nutrient content of this legume but limited information is available regarding their antioxidant activity and health relevant functionality. Hence, this study was carried out with the objective to determine the bioactivities, including antioxidant activities, nutraceutical properties and in vitro inhibitory potential against alpha-amylase, of the extracts of some newly developed desi and kabuli chickpea cultivars.

2) Materials and methods

2.1) Samples

Nine different cultivars of chickpea (Cicer arietinum L.) in their dried state were procured from ‘Sardar Vallabhbhai Patel University of Agriculture & Technology’, Meerut, India. These varieties included five Desi (dark brown) cultivars (PUSA-1103, PUSA-362, JG-62, K-850, and JG-74) and four Kabuli (white) cultivars (PUSA-1105, PUSA-1108, PUSA-1053, and PUSA-1088) of chickpea. The samples were cleaned by hand to remove dirt, grit and broken grains and then packed in air tight plastic containers.

2.2) Determination of color

Visual color of all cultivars of chickpea was measured by using X-rite colorimeter that was calibrated using a white reference standard tile, as described by Rangana, [12]. Three sets of readings were obtained per sample by rotating the cup each time. In this coordinate system, the ‘L’ value is a measure of lightness/brightness, ranging from 0 (black) to 100 (white), the ‘a’ value is a measure of greenness/redness, ranging from -60 (green) to +60 (red) and ‘b’ value is a measure of bluishness/yellowishness, ranging from -60 (blue) to +60 (yellow). The derived color function or chromaticity $C_{ab}$ (Chroma or Chromaticity) was calculated using the following formula Chromaticity = $[(a)^2 + (b)^2]^{1/2}$ as given by Fugita et al., [13].

2.3) Determination of the total polyphenolic content

The total polyphenolic content (TPC) of the aqueous methanolic extract of raw chickpea seeds was determined according to the method of Folin-Ciocalteu method [14]. One mL aliquot of the sample extract was taken in a test tube. Thereafter 5 mL of diluted folin ciocalteu reagent (1:10 with distilled water) and 4 mL sodium carbonate solution (7.5%, w/v) were added sequentially to each tube. Soon after mixing, the test tubes were placed in the dark for 60 minutes at room temperature and the absorbance was monitored by UV-VIS spectrophotometer (model-Evolution600) at 765 nm against blank as standard. A standard curve was prepared with “Gallic acid” and results were expressed in terms of mg per 100g of polyphenol present in the sample. Samples were analyzed in triplicates and mean was calculated.

2.4) Determination of total flavonoid content

The total flavonoid content of the chickpea seed extracts was determined according to the method of Boateng et al., [15]. Ethanolic extract (2 mL) was mixed with 150 µL of 5% NaNO$_2$. After 5 minutes, 150 µL of 10% AlCl$_3$ was added. After 10 minutes interval 1 mL of 1M NaOH and 1.2 mL of distilled water were added in this mixture. The mixture was shaken vigorously and after 10 minutes incubation absorbance was read at 510 nm by spectrophotometer. A calibration curve was prepared using a standard solution of quercetin (0.05-0.5 mg/mL). Samples were analyzed in three replications and results were expressed as mg quercetin equivalents / g (QE) of sample (dry basis).

2.5) Determination of total tannin content

Tannin content in chickpea was determined as described by Sadasivum & Manickam, [16]. 0.5 g of the powdered chickpea flour was taken in 250 mL conical flask and 75 mL distilled water was added. Flask was gently boiled for 30 minutes. After boiling, all the contents present in flask were centrifuged at 2,000 rpm for 20 min and the supernatant was collected in 100 mL volumetric flask and volume was made up to the mark. Thereafter 1 mL of the sample extract was carefully transferred to another 100 mL volumetric flask containing 75 mL distilled water, 5 mL of Folin-Denis reagent and 10 mL of sodium carbonate solution and further diluted to 100 mL with distilled water. The mixture was shaken properly and color developed was measured at 700 nm after 30 minutes of incubation period. Tannic acid was used to make standard curve. The results were expressed as mg/100g dry weight basis tannin present in the sample.

2.6) Percent (%) antiradical activity quantification

The per cent (%) antiradical activity was determined by the DPPH assay according to the method reported by Sanja et al., [17], with slight modification. 10 mg of chickpea seed flour was mixed with 10 mL acidified methanol. The sample solution was heated at 40 °C in water bath for 20 min. The resulting mixture was centrifuged at 2500-3000 rpm for 20 min. Extract for each variety was thus obtained in triplicate. To evaluate the reduction of the DPPH radical 100 µL of sample extract was taken in the test tube and diluted to 2.9 mL with methanol (pure). Thereafter this sample mixture was mixed with 150 µL of DPPH solution (4.3mg in 3.3mL methanol) which also served as a control with same concentration. The resulting sample solution was incubated at room temperature in the dark for 15 min. The mixture was shaken and decrease in absorbance was measured at 515 nm with the help of UV/VIS spectrophotometer after 15 min. The per cent (%) antiradical activity was calculated by following formula:-

\[
\text{Per cent (%) Antiradical activity} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100
\]

Where, control absorbance is the absorbance of the DPPH solution without the extract.

2.7) Determination of reducing capacity

The determination was carried out as described by Okty et al., [18]. Briefly, 2 mL of chickpea seeds extract was mixed
with phosphate buffer (2.5 mL, pH, 6.6) and potassium ferricyanide (2.5mL,1%, w/v), the mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid solution (10%) was added to the mixture, which was then centrifuged at 10,000g for 10 min. The upper layer of solution (2.5 mL) was mixed with deionised water (2.5 mL) and Ferric chloride solution (0.5 mL, 0.1%, w/v) and the absorbance of the mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The measurement was compared to a standard curve of prepared ascorbic acid (AA) solution, and the final results were expressed as micromoles of ascorbic acid equivalents (AAE) /g of flour.

2.8) Metal chelating (Fe²⁺) activity
The metal chelating activity of chickpea seeds sample was assessed as described by Sharma and Gujral, [19]. Extract (0.5 mL) was mixed with 50 µL of ferrous chloride and 1.6 mL of 80% methanol was added. After 5 min, the reaction was initiated by the addition of 5 mL/L ferrozone (100 µL) and the mixture was shaken on a vortex. The mixture was incubated at room temperature (25 °C) for 10 min. The absorbance of solution was measured at 562 nm on a spectrophotometer. The chelating activity of the extract for Fe²⁺ was calculated as follows:

Iron (Fe²⁺) chelating activity (%) = \{1 - (Abs. of sample / Abs. of control)\} × 100

2.9) Determination of ferric reducing antioxidant power (FRAP)
The FRAP was performed according to methods described by Sutharut and Sudarat [20]. Briefly, freshly prepared FRAP reagent consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃ in a ratio of 10:1:1 (v/v/v). The 200 µL of methanolic extract of each sample was mixed with 1.3 mL of the FRAP reagent and after 30min of incubation at 37°C, absorption was measured at 595 using a spectrophotometer. The absorbance changes in the test mixture were compared to those obtained from standard mixture of ferrous sulphate (FeSO₄. 7H₂O) (0.1 mmol/L – 1.0 mmol/L). FRAP values, expressed as mmol of Fe (II) equivalent / g flour.

2.10) Alpha (α)-amylase inhibition activity
The determination was done as described by Vadivel et al., [21]. 0.5 g of seed sample extracted for 2 h with 2.5 mL of 90% methanol. 100 µL of methanolic extract of three replicates were mixed with 100µL of 0.02M sodium phosphate buffer (pH 6.9) and 100 µL of α-amylase solution (0.5mg of α amylase dissolved in 1 mL of 0.006M NaCl) which was pre incubated at 25 °C for 10 min. Then 100 µL of 1% starch solution was added and further incubated at 25 °C for 30 min. The reaction was stopped by the addition of 1.0 mL of dinitro salicylic acid (DNS) reagent. The test tubes were then incubated in a running boiling water bath for 5 min and then cooled at room temperature. The reaction mixture was then diluted 10 times with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract and the per cent (%) α-amylase inhibition activity was calculated as follows:

\[
\text{Per cent (%) inhibition} = \left(\frac{\text{Control absorbance - extract absorbance}}{\text{Control absorbance}}\right) \times 100
\]

Where, control absorbance is the absorbance of the buffer instead of sample extract.

3) Statistical analysis
Analyses were performed in triplicates. Statistical analysis was performed using software programme SPSS for windows (version 7.5). Analysis of variance (ANOVA) was conducted and Duncan’s multiple range tests were used to determine the significant differences using the probabilities of 0.05. Pearson correlation test was applied to determine the linear correlation among the variables.

4) Results and discussion
4.1) Color values of desi and kabuli chickpea cultivars
Several recent studies have reported that legumes with dark seed coat such as black soyabean, red kidney bean, pinto bean and lentils possessed a high antioxidant activity due to the presence of high amount of phenolic compounds, mainly anthocyanins and tannins [22, 23]. Chickpea seed color ranged from cream to dark brown. The L, a, b and chromaticity values of chickpea seed flours are presented in Table 1. The color value L of a sample indicates its lightness. It has been reported that as L value of the sample decreases, the opaqueness of the sample increases. The

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>L ± SD</th>
<th>a ± SD</th>
<th>b ± SD</th>
<th>Chromaticity (C',a,)* ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-850</td>
<td>28.39± 0.48d</td>
<td>1.34 ±0.16b</td>
<td>9.94±0.19g</td>
<td>9.33±0.68e</td>
</tr>
<tr>
<td>JG-62</td>
<td>26.41± 0.18a</td>
<td>2.10± 0.21c</td>
<td>7.94±0.75a</td>
<td>9.38±0.74f</td>
</tr>
<tr>
<td>JG-74</td>
<td>28.82± 0.13e</td>
<td>1.32±0.14a</td>
<td>8.10±0.21b</td>
<td>10.67±0.11i</td>
</tr>
<tr>
<td>PUSA-362</td>
<td>27.61± 0.19c</td>
<td>2.98±0.10h</td>
<td>10.09±0.20h</td>
<td>8.42±0.22b</td>
</tr>
<tr>
<td>PUSA-1103</td>
<td>26.93 ± 0.17b</td>
<td>2.76 ±0.17g</td>
<td>9.93±0.15ef</td>
<td>8.25±0.71a</td>
</tr>
<tr>
<td>Kabuli type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUSA-1108</td>
<td>32.05± 0.31i</td>
<td>2.41±0.10f</td>
<td>9.91±0.34c</td>
<td>9.00±0.11d</td>
</tr>
<tr>
<td>PUSA-1088</td>
<td>29.16± 0.27f</td>
<td>1.76 ±0.34d</td>
<td>9.19±0.56d</td>
<td>8.56±0.10c</td>
</tr>
<tr>
<td>PUSA-1105</td>
<td>32.64±0.36h</td>
<td>1.66±0.25c</td>
<td>9.96±0.55g</td>
<td>10.08±0.10g</td>
</tr>
<tr>
<td>PUSA-1053</td>
<td>30.12±0.15g</td>
<td>1.66 ±0.32e</td>
<td>8.40±0.12c</td>
<td>10.12±0.15h</td>
</tr>
</tbody>
</table>

Results are expressed on dry weight basis
Values are expressed as mean values of three replications ± standard deviation
L value denotes lightness of the seed samples
a value denotes redness of the seed samples
b value denotes yellowness of the seed samples
(C',a) Chromaticity values denote color functions of the seeds samples
* Means followed by same superscript with in a column do not differ significantly (p≤0.05).

Table 1. Color values of desi and kabuli chickpea cultivars
a and b value indicate the intensity of red and yellow in the sample, respectively. Significant differences (p<0.05) in L and b parameters of color were found between desi and kabuli chickpea cultivars. Similar observations were found in other legumes also [24]. L values (Lightness) was higher in kabuli cultivars than desi chickpea cultivars. Desi cultivars being more pigmented and had lesser L value. The chromaticity value C_{ab} indicated that the desi cultivars of chickpea are perceived as more intense than the kabuli cultivars.

4.2) Total polyphenol content (TPC) of chickpea cultivars

Polyphenolic compounds have been proven to exhibit many health protective effects, having received most attention [25]. Phenolic compounds present in food ingredients such as cereals, legumes and vegetables are demonstrated to exhibit potential antioxidant [26], antimicrobial [27], anticancer [28], anti-obesity [29], antidiabetic and anti-hypertensive [30] as well as anti-mutagenic properties [31]. Epidemiological studies have also been suggested positive role played by phenolics in the alleviation of oxidative stress and prevention of free-radical mediated diseases [32]. The total polyphenol content (TPC) in the selected chickpea cultivars ranged from 0.15 mg QE/g of flour to 0.36 mg QE/g of flour (Table 2). Desi chickpea cultivars ranged from 0.15 mg QE/g of flour to 0.36 mg QE/g of flour (Table 2). Desi chickpea cultivars being more pigmented and had lesser L value. The chromaticity value C_{ab} indicated that the desi cultivars of chickpea are perceived as more intense than the kabuli cultivars.

Table 2 TPC, phytate, tannin and flavonoid content of desi and kabuli types of chickpea cultivars

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Desi type</th>
<th>TPC ± SD (mg/100g)</th>
<th>Tannin± SD (%)</th>
<th>Flavonoid± SD (mgQE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-850</td>
<td></td>
<td>255±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.36±0.15&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>JG-62</td>
<td></td>
<td>245±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.01&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.31±0.16&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>JG-74</td>
<td></td>
<td>223±1.52&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.17±0.01&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.29±0.11&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUSA-362</td>
<td></td>
<td>212 ± 0.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.18±0.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.34±0.12&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUSA-1103</td>
<td></td>
<td>203 ±3.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.18±0.01&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.34±0.10&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kabuli type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUSA-1108</td>
<td></td>
<td>178 ± 1.00&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.12±0.02&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.25±0.34&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUSA-1088</td>
<td></td>
<td>173 ±1.52&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.11±0.01&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.21±0.56&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUSA-1105</td>
<td></td>
<td>138 ± 1.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.06±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.15± 0.18&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUSA-1053</td>
<td></td>
<td>101 ± 1.60&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.18± 0.10&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed on dry weight basis.

<sup>*<sup>Values are expressed as mean values of three replications ± standard deviation</sup> <sup>Means followed by same superscript within a column do not differ significantly (p>0.05).<sup></sup></sup>

4.3) Total flavonoid content of desi and kabuli chickpea cultivars

Flavonoids are considered to be primary antioxidants and they act as free radical acceptors and chain breakers in foods [41]. Flavonoids have generated interest because of their broad human health promoting and synergistic effects with other antioxidants [23]. The position and the degree of hydroxylation is of primary importance in determining the antioxidant activity of phenols. The antioxidant mechanism of flavonoids, may also result from the interaction between flavonoids and metal ions especially iron and copper [42]. The total flavonoid content in selected desi and kabuli chickpea cultivars ranged from 0.15 mg QE/ g of flour to 0.36 mg QE/g of flour (Table 2). Desi chickpea cultivars with darker seed coat had significantly (p<0.05) higher total flavonoid content than kabuli cultivars of chickpea. Among desi cultivars K-850 had the highest flavonoid content (0.36 mg QE/ g of flour) followed by PUSA-1103 (0.34 mg QE/g of flour) and JG-62 (0.31 mg QE/g of flour). Studies have shown that legumes with darker seed coats have relatively higher total flavonoid content in comparison to those with lighter colored seed coats [43]. Boetang et al., [15] investigated the total flavonoid content in pinto beans and kidney beans. He reported that pinto beans had 0.614 mg QE/g and kidney beans had 0.845 mg QE/g of flavonoids, respectively. Heimler et al.,[44] reported similar values for total flavonoids in dry beans. Oomah et al., [45] reported values of 0.24 and 0.26 mg CE/g for flavonoid content in pinto beans and red kidney beans respectively. The high flavonoid levels in the beans may be due to their high anthocyanidin contents. Total flavonoid (0.29 mg QE/g) content in black soybeans was
found to contain higher flavonoid than yellow soybeans (0.45 mg QE/g) [46]. Total flavonoid contents in legumes varied greatly.

4.4 Total tannin content in selected chickpea cultivars

Tannins are generally produced from the polymerization of phenolics. They possess variety of molecular structures. They are predominantly divided into hydrolysable and condensed proanthocyanidins. Tannins are biologically active compounds and may have beneficial or adverse health effects. To investigate the potential for the antioxidant activity the condensed tannins from different chickpea cultivars were determined and results expressed as per cent (%) tannic acid content in the samples as presented in Table 2. In the present study total tannin content ranged from 0.17% to 0.21% in desi chickpea cultivars and 0.06% to 0.12% in kabuli cultivars of chickpea. It was being highest in desi cultivar of chickpea namely K-850 (0.21%) followed by JG62 (0.20%) and lowest in PUSA-1105 (kabuli type). Studies have shown that comparison to other legumes lentil class exhibited the highest average level of condensed tannin (approximately 6 mg) followed by common beans (approx. 4 mg), black soyabeans (approx.2 mg) and green and yellow pea (approx. less than 0.2 mg)[47]. These values were lower in concentration as reported for beach pea seeds ranged from 7.19 mg per g in fresh green seeds to 11.7 mg per g in fully mature dark green seeds. It has been previously reported by other workers also that tannins are seen more in dark colored seeds than pale yellow colored seeds because there is higher degree of polymerization of existing polyphenolic compounds due to maturation and play an important role in the defense system of seeds that are exposed to oxidative damage by many environmental factors. Other factors like plant type, age of plant, stage of development and environmental conditions govern the tannin content in plants.

4.5 Per cent (%) antiradical activity of desi and kabuli chickpea

The DPPH method to assess the radical scavenging activity of natural compounds received much attention due to its fast and reliable results. This method is based on the reduction of alcoholic DPPH solution by hydrogen donating antioxidant compound into its non radical form (DPPH-H). The free radical scavenging activity of aqueous methanolic chickpea seeds extract is presented in Table 3. The decrease in absorbance of the DPPH radical caused by antioxidant was due to scavenging of the radicals by hydrogen donation is visually noticeable as a color change from purple to yellow [48]. Extract obtained from desi cultivars of chickpea seeds, K-850 (88%) showed good percent (%) antiradical activity followed by JG-62 (87%) and JG-74 (86%). In general the antioxidant activity of desi cultivars of chickpea was found better than kabuli cultivars of chickpea. The relative higher antioxidant activity of K-850 and JG-62 (desi cultivars) compared to other cultivars of chickpea could be attributed to their higher bioactive phytochemical contents viz. poly phenols, flavonoid, tannin and phytate contents. Per cent (%) antiradical activity of desi and kabuli chickpea cultivars is higher than the previous reports on an under-utilized legume, *Macuna pruriens* (50%)[49] and certain common legumes like *Phaseolus vulgaris* var. Bayo Victoria (40%) [50], *Vigna radiata* (25%) [51] and soybean (44%) [52]. These values are in line with certain other legumes like- *Vicia faba* (60%) [53] and *Caesalpinia bonducella* (69%) [54] etc. Djordjevic et al., [55] examined antioxidant activity of four legumes by DPPH method and he found that among examined legumes soybean showed the weak effects (35%) followed by mung beans (64.6%), lentils (67%) and red kidney beans (69%). These findings agree with many earlier reports and confirm that there is correlation between total polyphenols content and antioxidant activity of some plant foods. The antiradical activity of phenolic is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts (O-H bonds, dissociation energy, resonance delocalization, steric hindrance derived from bulky groups) of their chemical structure. Potential antiradical activity of both desi and kabuli chickpea seed samples used in this study is most likely due to the hydrogen donating capacity of the phenolic compounds and might protect against various free radical related disorders.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Desi type</th>
<th>Antiradical activity ± SD (%)</th>
<th>Reducing capacity ± SD (µmolAAE g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-850</td>
<td>84±0.18°</td>
<td>35±1.00°هد</td>
<td>34±1.52°هد</td>
</tr>
<tr>
<td>JG-62</td>
<td>87±0.15°هد</td>
<td>33±1.52°هد</td>
<td>31±1.00°هد</td>
</tr>
<tr>
<td>JG-74</td>
<td>86±0.20°هد</td>
<td>32±2.08°هد</td>
<td>30±1.52°هد</td>
</tr>
<tr>
<td>PUSA-362</td>
<td>85±0.25°هد</td>
<td>84±0.02°هد</td>
<td>29±2.08°هد</td>
</tr>
<tr>
<td>PUSA-1103</td>
<td>85±0.28°هد</td>
<td>68±1.91°هد</td>
<td>24±1.00°هد</td>
</tr>
<tr>
<td>Kabuli type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUSA-1108</td>
<td>86±0.22°هد</td>
<td>67±0.88°هد</td>
<td>23±1.52°هد</td>
</tr>
<tr>
<td>PUSA-1088</td>
<td>77±1.02°هد</td>
<td>67±0.88°هد</td>
<td>23±1.52°هد</td>
</tr>
<tr>
<td>PUSA-1105</td>
<td>68±1.91°هد</td>
<td>67±0.88°هد</td>
<td>23±1.52°هد</td>
</tr>
<tr>
<td>PUSA-1053</td>
<td>67±0.88°هد</td>
<td>67±0.88°هد</td>
<td>23±1.52°هد</td>
</tr>
</tbody>
</table>

* Values are expressed as mean values of three replications ± standard deviation
* Means denoted by same superscript within a column do not differ significantly (p≤0.05).

4.6 Reducing capacity of selected chickpea cultivars

The reducing power is also an indicator of antioxidant activity. The electron donor compounds are considered as reducing agents and can reduce the oxidized intermediates of the lipid peroxidation reactions therefore they may be primary or secondary antioxidants [56]. Reducing power is
a sensitive method for the semi-quantitative determination of dilute concentrations of poly phenolics, which participate in the redox reaction [44]. In the present study, reducing capacity against ascorbic acid was determined and the results are presented in Table 3. The reducing capacity of desi and kabuli chickpea cultivars ranged from 23 to 35 \( \mu \text{mol AAE/g of flour} \). K.850 (desi cultivar) showed highest reducing capacity, while PUSA 1053 showed lowest reducing capacity. Liyana-Pathirana and Shahidi [57] reported a reducing power ranging from 99 to 131 \( \mu \text{mol AAE/g of flour} \) in different wheat cultivars. Similar values were also reported by Oboh et al., [58] for soybean seed extracts.

4.7) Metal chelating activity
Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress [59]. The interaction of ferrous ion (Fe\(^{2+}\)) with ferrozine produces a dark color complex that is decreased by the action of metal chelator compounds present in the reaction mixture. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe\(^{2+}\) possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [60]. The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups [61]. The metal chelating potential of different cultivars of chickpea is shown in Table 4. As observed all the extracts of chickpea seeds exhibited good metal chelating activity. Among desi cultivars of chickpea K850 registered higher metal chelating activity (66%) followed by JG62 (55%) and JG74 (53%) while in kabuli cultivars PUSA 1108 had highest metal chelating activity (36%) in comparison to PUSA1053 which had lowest (27%). Our results falls within the range of the values reported for Mungbean (92%), red sorghum (80%), and foxtail millet (59%). These results indicated that the seed extracts of desi and kabuli C. arietinum cultivars have a potency to donate electron to deactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

Table 4. Metal chelating activity and ferric reducing antioxidant power (FRAP) of desi and kabuli chickpea cultivars

<table>
<thead>
<tr>
<th>Cultivars Desi type</th>
<th>Metal chelating activity ± SD (%)</th>
<th>FRAP ± SD (mmol Fe (II) Eq.g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-850</td>
<td>67±2.08</td>
<td>3.20±0.05c</td>
</tr>
<tr>
<td>JG-62</td>
<td>55±3.05c</td>
<td>6.18±0.09d</td>
</tr>
<tr>
<td>JG-74</td>
<td>53±3.21e</td>
<td>3.06±0.05a</td>
</tr>
<tr>
<td>PUSA-362</td>
<td>51±1.52e</td>
<td>5.21±0.03f</td>
</tr>
<tr>
<td>PUSA-1103</td>
<td>42±2.00d</td>
<td>4.94±0.04e</td>
</tr>
<tr>
<td>Kabuli type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUSA-1108</td>
<td>36±2.00e</td>
<td>3.20±0.05d</td>
</tr>
<tr>
<td>PUSA-1088</td>
<td>33±1.00bc</td>
<td>2.70±0.02b</td>
</tr>
<tr>
<td>PUSA-1105</td>
<td>32±2.00b</td>
<td>2.07±0.02a</td>
</tr>
<tr>
<td>PUSA-1053</td>
<td>27±1.52a</td>
<td>2.03±0.04a</td>
</tr>
</tbody>
</table>

* Means denoted by same superscript with in a column do not differ significantly (p≤0.05).
* Values are expressed as mean values of three replications ± standard deviation.

4.8) Ferric reducing antioxidant power (FRAP)
Inactivation of oxidants by antioxidants or reductants can be described as redox reaction in which one reactive species (oxidant) is reduced at the expense of antioxidant [62]. The FRAP assay measures the antioxidant effect of any substance in the reaction medium in terms of its reduction ability. Antioxidant potential of Methanolic extracts of desi and kabuli chickpea seed extracts were determined by their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The ferric reducing antioxidant potential of desi chickpea cultivars ranged from 3.06 to 6.18 mmol of Fe (II) equivalent per g flour and for and kabuli cultivars it was ranged between 2.03 to 3.20 mmol of Fe (II) equivalent/g flour (Table 4). Higher FRAP value was exhibited by JG62 (6.18 mmol of Fe (II) eq/g). These values were higher than in earlier reports on Vigna vexillata (1.96 mmol Fe (II)/g extract [63] and red sweet pepper (6.32 mmol Fe(II)/g extract)[64]. Our results of FRAP were also in good agreement with that of (0.57 to 0.65 mmol Fe/ g) reported previously for peas [65] and pinto bean (1.27 mmol Fe(II)/g) [36]. Xu et al.,[66] reported that lentil exhibited highest FRAP values (11.79 mmol Fe(II)/100 g) followed by black soybeans (9.43 mmol Fe(II)/per100 g), common beans (5.83 mmol Fe(II)/100/g), and the other 3 groups of yellow soybeans and yellow peas and green peas (all were below 1.5 mmol Fe(II)/100 g). Therefore, the FRAP results indicate that these chickpea varieties suppose to have a potential antioxidant activity.

4.9) \( \alpha \)-Amylase inhibition activity
Pancreatic \( \alpha \)-amylase is a key enzyme in the digestive system and catalyzes the initial step in the hydrolysis of starch [67] which is a principal source of glucose in the diet. Today dietary solutions for the management of type II diabetes have supreme research priority. Considering the diet linked challenges of type II diabetes mellitus, consumption of foods rich in \( \alpha \)-amylase and \( \alpha \)-glucosidase inhibitors acquires more attention and is extensively investigated [39]. \( \alpha \)-Amylase inhibitors are starch blockers.
which affect the enzyme activity thus playing a vital role in reducing blood sugar [68]. The inhibitory potential of the different cultivars of chickpea are shown in Fig.1. As observed all the extracts of chickpea seeds exhibited good alpha-amylose inhibitory activity. K 850 possessed highest alpha-amylose inhibiting activity (62%) while PUSA 1105 and PUSA 1053 had lowest (52% and 49%, respectively) alpha-amylose inhibiting activity. This value is lower than Mucuna pruriens seeds (87%) but comparable with that of mung bean (65%) and some cereal grains such as wheat, buckwheat, corn and oats (38 – 55%), Foxtail millet (32%), Proso millet (55%) and finger millet (55%) [68, 12].

Results of the present study are in agreement with those of previous researches, which have demonstrated that phytochemical derived from plant have moderate to high alpha amylose inhibiting activity [69]. In this way these natural enzyme inhibitors would likely offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia. Therefore, these results indicate that chickpea cultivars investigated in this study have the potential to contribute to the management of type 2 diabetes, because of their potential inhibition against α-amylose.

![Figure 1: Alpha amylase inhibition activity of different cultivars of chickpea. Values are presented as mean of three replications ± Standard deviations.](image)

**Table 5.** Correlation between TPC, antioxidant activities and color values of chickpea cultivars

<table>
<thead>
<tr>
<th>Cultivars of chickpea</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>Chromaticity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>-0.66*</td>
<td>0.074</td>
<td>0.019</td>
<td>0.236</td>
</tr>
<tr>
<td>ARA</td>
<td>-0.63*</td>
<td>0.276</td>
<td>0.039</td>
<td>0.361</td>
</tr>
<tr>
<td>Reducing Capacity</td>
<td>-0.68*</td>
<td>-0.108</td>
<td>0.044</td>
<td>-0.288</td>
</tr>
</tbody>
</table>

TPC is the abbreviation of Total polyphenol content
ARA is the abbreviation of Antiradical activity
*Correlation is significant at a 0.05 level (2 tailed)

**4.10 Correlation analysis among bioactive compounds, color values, per cent (%) antiradical activity and reducing capacity of chickpea cultivars**

The correlation coefficients among TPC, per cent (%) antiradical activity, reducing capacity and color values of chickpea seed flours are shown in Table 5. Correlation analysis showed that TPC and antiradical activity were negatively correlated with lightness (L value) ($r^2 = 0.669$, $p < 0.05$ and $r^2 = 0.633$, $p < 0.05$, respectively) and positively correlated with a value ($r^2 = 0.074$ and $r^2 = 0.276$, respectively). Similar observations were also found by other investigators in various seed legumes [28,67]. Our result reveals that darkness and high color intensity in chickpea seeds were associated with more TPC and antiradical activity values than light colored seeds and this further substantiate that desi cultivars had higher TPC and antioxidant activity in comparison to kabuli types. It is assumed that more darkness/redness (a value) indicate that these cultivars are highly pigmented and therefore could be possibly used as a marker for selection of polyphenol rich cultivars.

Correlation between TPC, per cent (%) antiradical activity, reducing capacity, metal chelating activity, FRAP and alpha amylase inhibiting activity is presented in Table 6. All these parameters were positively and significantly correlated with each other. Earlier researches also confirm the correlation of tested parameters in barley, oats, buckwheat and wheat [66, 69] and in the hulls of fava beans, broad beans, lentils and peas etc. Correlation analysis between TPC and per cent (%) antiradical activity which was assessed by DPPH method revealed significant and positive correlation ($r^2 = 0.934$, $p < 0.01$) while in the case of reducing capacity, metal chelating activity and alpha amylase inhibiting activity it was found 0.977, 0.684 and 0.953 respectively. Rhode et al.,[69] reported good correlation between alpha amylase inhibiting activity and TPC. He stated that plant phenolic substances react with protein/enzymes, influencing their physicochemical properties and in vitro enzymatic activity. The reason behind it is maybe due to the fact that phenolics mostly exist in the form of aglycosides and aglycones. Since the enzyme operates in the aqueous phase, direct enzyme inhibitor interaction is expected. Specifically for alpha amylase author affirm that the anti enzyme activity depends on the concentration and on the number and position of the hydroxyl groups of the phenolic compound. Similarly correlation analysis between total flavonoid content and per cent (%) antiradical activity revealed significant and positive correlation ($r^2 = 0.910$, $p < 0.01$). While on the other hand correlation analysis of total flavonoid content with reducing capacity and alpha amylase inhibiting activity was found to be 0.890 and 0.895which was also positive and significant (Table 6).

The correlation analysis between tannins and per cent (%) antiradical activity is also presented in (Table 6). Unlike the laboratory-generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. Correlation analysis between total tannin content and per cent (%) antiradical activity revealed significant and positive correlation ($r^2 = 0.930$, $p < 0.01$), while on the other side correlation analysis between total tannin content and reducing capacity was found to be 0.950. Amarcowicz et al., [70] found good correlation between tannin and radical scavenging activity in beach peas, canola hulls, and fava beans. Duenas et al. [43] also reported a significant correlation between DPPH assay and proanthocyanidins present in moca fruits ($r^2 = 0.961$). Correlation analysis of activity was found to be 0.683, 0.753 and 0.932 which were in line with some early findings.
Table 6: Correlation between phenolics and antioxidant properties of chickpea cultivars

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH</th>
<th>Reducing capacity</th>
<th>Metal chelating activity</th>
<th>FRAP</th>
<th>α-amylase inhibiting activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPC</td>
<td>0.934*</td>
<td>0.978*</td>
<td>0.934*</td>
<td>0.633**</td>
<td>0.953*</td>
</tr>
<tr>
<td>TFC</td>
<td>0.912*</td>
<td>0.896*</td>
<td>0.847*</td>
<td>0.726**</td>
<td>0.896*</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.935*</td>
<td>0.956*</td>
<td>0.908*</td>
<td>0.753**</td>
<td>0.932*</td>
</tr>
</tbody>
</table>

*Correlation is significant at 0.01 (p two-tailed).
**Correlation is significant at 0.05 (p two-tailed).

4FRAP is the abbreviation of Ferric reducing/antioxidant potential

[43] also reported a significant correlation between DPPH assay and polyanthocyanidins present in mocan fruits (r²=0.961). Correlation analysis of activity was found to be 0.683, 0.753 and 0.932 which were in line with some early findings.

5) Conclusion

In conclusion, findings of this study indicate that varietal differences exist in the ‘bioactive compound’ contents of chickpeas. The results of this study therefore show that desi cultivars of chickpea can be used as a functional food in addition to their potential role of inhibiting alpha-amylase in vitro than kabuli cultivars. Their relatively higher free radical scavenging activity or per cent antiradical activity could be a result of their relative higher total polyphenol content, flavonoid and tannin content. It is therefore concluded that these desi cultivars of chickpea in general and K-850 in particular could contribute significantly in the management and prevention of degenerative diseases associated with free radical damage, in addition to their traditional role of preventing protein malnutrition.

Acknowledgement

We are thankful to Prof G K Rai, Director IPS, University of Allahabad for providing all necessary facilities for this research work. Financial support given in the form of UGC- JRF Scholarship is also duly acknowledged to carry out this study.

Conflict of interest

Conflict of interest declared none.

References


Source of support: Nil; Conflict of interest: None declared