Comparison of cultivated and wild chickpea genotypes for nutritional quality and antioxidant potential

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Abstract Fifteen cultivated (Ten desi, five kabuli) and fifteen wild species of chickpea (*Cicer arietinum* L.) were compared for nutritional traits, antinutritional factors and antioxidant potential. The average crude protein content in desi, kabuli and wild species was found to be 25.31%, 24.67% and 24.30%, respectively; total soluble sugars in these genotypes were 38.08, 43.75 and 33.20 mg/g, respectively and total starch content in these genotypes was 34.43, 33.43 and 28.77%. Wild species had higher antioxidant potential as compared to cultivated genotypes due to higher free radical scavenging activity, ferric reducing antioxidant power and reducing power. Kabuli genotypes had lower antioxidant potential than desi genotypes. Desi genotype, GL 12021 had high crude protein and total starch content, lower phytic acid and saponin content and higher antioxidant potential. GNG 2171 had high crude protein and total soluble sugar content and lower tannin and phytic acid content. Kabuli genotype L 552 possessed high total soluble sugar and total starch content, high Zn and Fe content and lower tannin, saponin and trypsin inhibitor content. Wild species C. pin ILWC 261 had high crude protein, lower phytic acid and trypsin inhibitor content and higher DPPH radical scavenging activity and hydroxyl radical scavenging activity. The observed

diversity for quality traits in cultivated and wild genotypes can be further used.

Keywords Antioxidant potential · Chickpea · Cultivated genotypes · Quality traits · Wild species

Abbreviations

BAPNA *N*α-benzoyl-DL-arginine *p*-nitroanilide
DPPH (2,2-Diphenyl-1-picryl hydrazyl)
FRAP Ferric reducing antioxidant power
ROS Reactive oxygen species

Introduction

Chickpea (*Cicer arietinum* L.) is an important food crop both for human food as well as animal feed. It is the world's second largest grown pulse crop after beans. Chickpea is a cheap source of protein and ranked the fifth most valuable legume. India is the producer of 75% of world's chickpea production. The chickpea production has increased globally by 56% and in India by 55% during the decade of 2004–2013. Other major chickpea producing countries are Australia, Pakistan, Myanmar, Ethiopia, Mexico, Canada, USA, Tanzania and Malawi (Gaur et al. 2016). Year 2016 was declared as the international year of pulses by the United Nations General Assembly. The production of pulses in India was 17.56 MT in 2016 and India emerged as the biggest chickpea producer in the world with a production of 7.8 MT in 2016 (Kumar et al. 2018).

Chickpea is an outstanding source of nutritional constituents such as proteins, carbohydrates, vitamins (niacin and thiamine), minerals and unsaturated fatty acids (linolenic and oleic acids) (Heiras-Palazuelos et al. 2013).

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Popularity of chickpea in human diet is due to the balanced seed nutrients composition and its low price. People who cannot afford animal protein and those who are vegetarians mainly consume chickpea as a protein source in semi-arid regions. Cereals are rich in thiol containing amino acids (methionine and cysteine) and deficient in lysine whereas pulses are rich in lysine and deficient in methionine and cysteine. Due to this pulses are taken with addition of cereals for proper intake of essential amino acids (Reinkensmeier et al. 2015).

Cultivated chickpea are categorized into Desi and Kabuli types. The Desi (microsperma) types have pink flowers, anthocyanin pigmentation on stems, and seed coat is thick and coloured. The Kabuli (macrosperma) types have white flowers, lack anthocyanin pigmentation on stems, seeds are white or beige coloured having ram's head shape, seed coat is thin and a smooth seed surface. Geographical distribution of these chickpea types distinctly separate them as desi types which are mostly grown in Asia and Africa and account for up to 80–85% of the total chickpea area and kabuli chickpea types are grown mostly in West Asia, North Africa, North America and Europe (Gaur et al. 2016). Wild chickpea species are promising natural reservoir of potential genes for crop improvement due to their higher genetic variability and propagation in large range of habitats without human selection (Munoz et al. 2017).

Protein digestibility in legumes is affected by antinutritional factors such as phytate, raffinose family oligosaccharides, tannins, phenolic compounds, protease inhibitors (trypsin inhibitors and α amylase inhibitors) and saponins which are naturally occurring substances in edible seeds and impair absorption and nutritional utilization in humans by binding to proteins, minerals and vitamins (Soetan and Oyewole 2009).

Free radicals generated in many biochemical reactions cause functional and structural damage to nucleic acid, lipids, protein and cellular molecules and are mediators of many diseases in human body (Kumaran and Karunakaran 2007). Antioxidants in small quantities prevent the excessive collection of free radicals or reactive oxygen species (ROS) by retarding the oxidation of unsaturated fats which are easily oxidized. Pulses are also rich in natural antioxidants required for health and reduce the risk of diseases. Natural antioxidants are considered secure for the consumers than synthetic antioxidants such as butylated hydroxytoluene (BHT), which have carcinogenic effects. Desi and kabuli chickpea significantly differ at nutritional level due to differences with respect to physiochemical properties, protein digestibility, phenolic content and antioxidant activity (Heiras-Palazuelos et al. 2013). The present investigation has been formulated to compare desi, kabuli and wild chickpea types for quality traits so as to

identify cultivars with enhanced seed quality and this information can be further used to develop cultivars of desirable traits.

Materials and methods

Plant material

Thirty chickpea genotypes comprising of ten desi, five kabuli and fifteen wild species were procured from Pulses Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Different accessions of wild species, *Cicer judaicum* (C. jud), *Cicer pinnatifidum* (C. pin), *Cicer echinospermum* (C. ech) were also evaluated for quality traits. Foreign material and damaged seeds were removed from the sample. The seeds were crushed into fine powder using grinder and the powdered chickpea seeds were used for estimation of quality traits.

Extraction and determination of nutritional and antinutritional traits

Crude protein was estimated by Kjeldahl method. Seed powder (0.2 g) wrapped in Whatman No. 1 filter paper was put in the kjeldahl digestion tube and 3 g of digestion mixture (copper sulphate and potassium sulphate (1:10) followed by addition of 10 ml of conc. sulphuric acid. The digestion was carried out for 2 h in kjeldahl digester KELPLUS (KES 20L VADLS TS). After digestion, distillation was carried out in KELPLUS (CLASSIC-DX VATS (E) and then samples were titrated in titrator (Metrohm) using 0.1 N HCl. The nitrogen content determined was converted to protein content using conversion factor 6.25. Chickpea seed powder was extracted twice with 15 ml of 80% ethanol and 70% ethanol by keeping the tubes fitted with water condensers in boiling water bath for 20 min. The supernatants after each extraction were collected and pooled and total soluble sugars were estimated by using phenol sulphuric acid method as described previously by Sekhon et al. (2017). The collected dried soluble sugar free residue was used to extract and estimate starch using perchloric acid. Bound fructose of sucrose and raffinose family oligosaccharides was estimated by using resorcinol–HCl after destroying free fructose by boiling with 30% NaOH. Seed powder was digested with concentrated nitric acid and perchloric acid (4:1) till clear solution was obtained, volume was made to 25 ml with deionized distilled water and it was filtered using Whatman filter paper No. 1. Elements such as iron, zinc, calcium, magnesium, copper and manganese were estimated using Atomic Absorption Spectrophotometer (Perkin Elmer).

The instrument was calibrated by using specific standards for each element.

Antinutritional factors such as phytic acid, tannins, trypsin inhibitor and saponins were determined from powdered chickpea seeds by the methods as described previously by Sekhon et al. (2017). Phytic acid was extracted with 1.2% HCl and precipitated with ferric chloride and inorganic phosphorus was estimated. Tannins were estimated from seed powder by Folin-Denis reagent. Saponins were extracted with acetone and later with methanol from the powdered seed powder and estimated by using reagent anisaldehyde (0.5 ml) mixed with (99.5 ml) ethyl acetate and concentrated sulphuric acid and concentration was calculated from standard curve prepared by using saponin (0–40 µg) as a standard. Trypsin inhibitor was extracted from powdered seeds with 0.1 M phosphate buffer (pH 7.5) containing 0.1 M NaCl and centrifuged at 10,000×g for 20 min. The supernatant was incubated at 80 °C for 20 min and then again centrifuged at 10,000×g for 20 min. The supernatant was used for assaying the bovine trypsin inhibition and *N*α-benzoyl-DL-arginine *p*-nitroanilide (BAPNA) was used as a substrate. One inhibitor unit is defined as the quantity of inhibitor which causes 50% inhibition of trypsin activity.

Determination of antioxidant potential

The seed powder was refluxed with 80% methanol for 1 h at 80 °C. The mixture was vortexed for two hours and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and the pellet was re-extracted twice with 80% methanol. The pooled supernatants were used to measure antioxidant capacity of chickpea genotypes. Total phenols, flavonols, total reducing power, ferric reducing antioxidant power and free radical scavenging activities such as DPPH (2,2-diphenyl-1-picryl hydrazyl) free radical, hydroxyl radical, superoxide anion radical and nitric oxide radical were estimated in seeds of cultivated and wild species by the methods as described previously by Sekhon et al. (2017).

Statistical analysis

Mean and standard deviation were calculated. The results were analyzed by Tukey's test. Software SPSS pc version 20.0 was used to identify correlation between the nutritional, antinutritional and antioxidant potential in chickpea genotypes. Equal class distribution method was used to categorize chickpea genotypes into three equal groups (low, medium and high) for quality traits.

Results and discussion

Nutritional constituents

Cultivated chickpea comprises two distinct seed types, desi and kabuli: desi seeds are generally brown or blackish brown while kabuli are of cream or beige colour. Due to these differences both desi and kabuli have different uses. We evaluated desi and kabuli chickpea genotypes to assess differences for nutritional quality and for antioxidant components so that potential donors can be identified for their use in breeding programmes to improve both desi and kabuli types for nutritional quality and antioxidant traits by transfer of desirable traits from potential donors. Similarly, it was assumed that wild species may possess some desirable nutritional and antioxidants which can be introgressed into cultivated chickpea. The variation in seed weight in desi, kabuli and wild species are presented in (Table 1). Seed weight of desi genotypes ranged from 7.19 g/100 seeds (GL 29095) to 13.69 g/100 seeds (GL 15017) with an average value of 9.07 g/100 seeds and in kabuli genotypes ranged from 15.16 g/100 seeds (L 552) to 22.97 g/100 seeds (GLK 14313) with an average value of 18.0 g/100 seeds. Wild species are smaller in size and their seed weight ranged from 0.76 g/100 seeds to 1.59 g/100 seeds with mean value of 0.93 g/100seeds. Kabuli genotype (GLK 14313) has the highest seed weight and wild species (*C. jud* 17148) has the lowest seed weight. Gaur et al. (2016) reported that seed weight of desi chickpea genotypes was found to be 10.2 g/100seeds and that of kabuli chickpea was 54.5 g/100 seeds. Seed weight, an extrinsic seed quality trait, also affects the reserve accumulation. Cooking time depends on seed size (weight and volume) and seed size is related to hydration and swelling capacity (Iqbal et al. 2006).

Legumes are known as 'meat of the poor people' because of their high protein content and are considered as staple food for those who cannot afford animal proteins or vegetarian by choice and for people affected by nutrition related health problems such as diabetes, obesity and over weight. Chickpea has protein quality better than other legumes and is a good source of dietary protein (Kaur and Singh 2007). The crude protein content in chickpea genotypes (Table 1) ranged from 23.03% (*C. jud* 17150) to 28.00% (GNG 2171). Crude protein content in desi genotypes was found in the range of 23.19 to 28.00% with the mean value of 25.31% and in kabuli genotypes it lied in the range of 23.15 to 25.97% with the mean value of 24.67%. In wild species, crude protein ranged from 23.03 to 26.82% with an average value of 24.30%. The chickpea genotypes with respect to crude protein were characterized into high, low and medium group using equal class distribution

Table 1 Distribution of seed weight (g/100 seeds), crude protein (%), total soluble sugar (mg/g), total starch (%), iron (mg/100 g), zinc (mg/100 g), calcium (mg/100 g), magnesium (mg/100 g), copper (mg/100 g) and manganese (mg/100 g) content in cultivated and wild chickpea genotypes

Genotypes	Seed weight	Crude protein	Total soluble sugar	Total starch	Zinc	Iron	Calcium	Magnesium	Copper	Manganese
GL 12021	8.42 ± 0.25 ^{gh}	27.69 ± 0.5 ^{ab}	39.06 ± 1.23 ^{cd}	35.62 ± 9.13 ^{bcd}	3.12 ± 0.21 ^{abc}	7.99 ± 0.12 ^{abcd}	62.01 ± 5.23 ^e	83.86 ± 6.05 ^{fg}	0.35 ± 0.13 ^{bed}	2.41 ± 0.13 ^{bcd}
GL 13037	11.83 ± 0.42 ^e	25.03 ± 0.28 ^{efgh}	52.73 ± 1.95 ^{ab}	34.71 ± 9.14 ^{cdef}	1.63 ± 0.18 ^{cd}	9.29 ± 0.70 ^{abc}	82.51 ± 7.66 ^e	87.03 ± 5.82 ^{efg}	0.30 ± 0.05 ^{bed}	1.96 ± 0.11 ^{efgh}
GL 13042	9.11 ± 0.17 ^{fg}	24.09 ± 0.72 ^{ijkl}	31.25 ± 1.54 ^{gh}	39.27 ± 9.14 ^a	2.00 ± 0.21 ^{bed}	7.83 ± 0.13 ^{abcd}	102.36 ± 15.94 ^{de}	84.91 ± 3.45 ^{fg}	0.40 ± 0.01 ^{bed}	2.28 ± 0.13 ^{cdefgh}
GL 14015	10.01 ± 0.28 ^f	23.19 ± 0.25 ^m	33.20 ± 1.95 ^{efg}	36.99 ± 4.57 ^b	2.12 ± 0.18 ^{ghcd}	9.83 ± 0.19 ^a	40.83 ± 3.93 ^e	71.96 ± 11.39 ^g	0.21 ± 0.14 ^{bed}	2.73 ± 0.12 ^{bcd}
GL 15003	7.38 ± 0.07 ^h	25.94 ± 0.09 ^{cd}	27.34 ± 1.75 ^h	36.08 ± 4.57 ^{bcd}	2.93 ± 0.07 ^{abcd}	5.53 ± 0.15 ^{cd}	56.95 ± 8.85 ^e	76.42 ± 7.09 ^{fg}	0.26 ± 0.04 ^{bed}	3.49 ± 0.08 ^{abc}
GL 15017	13.69 ± 0.28 ^d	23.97 ± 0.16 ^{ijklm}	31.25 ± 1.66 ^{gh}	33.79 ± 9.14 ^{efgh}	2.42 ± 0.23 ^{abcd}	4.56 ± 1.75 ^d	55.11 ± 4.92 ^e	73.23 ± 7.23 ^{fg}	0.13 ± 0.05 ^d	2.06 ± 0.17 ^{defgh}
GL 29078	7.74 ± 0.14 ^h	23.72 ± 0.09 ^{klm}	37.10 ± 1.95 ^{cde}	31.51 ± 4.57 ^{ijk}	3.76 ± 0.12 ^{ab}	8.33 ± 0.13 ^{abcd}	53.78 ± 9.49 ^e	80.32 ± 2.22 ^{fg}	0.29 ± 0.01 ^{bed}	2.60 ± 0.04 ^{bcd}
GL 29095	7.19 ± 0.03 ^h	25.66 ± 0.22 ^{def}	35.15 ± 1.45 ^{efd}	31.51 ± 4.57 ^{ijk}	3.75 ± 0.10 ^{ab}	8.92 ± 0.54 ^{abc}	76.69 ± 6.56 ^e	92.86 ± 8.08 ^{efg}	0.33 ± 0.15 ^{bed}	2.26 ± 0.17 ^{cdefgh}
GING 2171	8.02 ± 0.43 ^{gh}	28.00 ± 0.38 ^a	54.68 ± 1.34 ^a	30.60 ± 4.57 ^{jk}	2.81 ± 0.29 ^{abcd}	5.80 ± 0.61 ^{abcd}	140.38 ± 14.46 ^{abcde}	145.12 ± 4.18 ^{abcde}	0.27 ± 0.06 ^{bed}	1.80 ± 0.16 ^{gh}
PBG 7	7.30 ± 0.14 ^h	25.85 ± 0.1 ^{de}	39.06 ± 1.27 ^{cd}	34.25 ± 22.84 ^{defg}	2.24 ± 0.24 ^{abcd}	7.24 ± 0.52 ^{abcd}	242.60 ± 13.57 ^{abc}	119.09 ± 4.23 ^{abcde}	0.18 ± 0.06 ^{cd}	2.12 ± 0.13 ^{defgh}
Mean	9.07 ± 0.22	25.31 ± 0.28	38.08 ± 1.61	34.43 ± 6.19	2.68 ± 0.18	7.53 ± 0.38	91.32 ± 8.06	91.48 ± 5.98	0.27 ± 0.07	2.37 ± 0.12
L 552	15.16 ± 0.26 ^c	24.13 ± 0.22 ^{ijkl}	52.73 ± 1.95 ^{ab}	36.53 ± 18.27 ^{bcd}	4.05 ± 0.41 ^a	9.87 ± 0.94 ^a	56.44 ± 3.90 ^e	132.27 ± 6.82 ^{abcde}	0.38 ± 0.10 ^{bed}	2.88 ± 0.19 ^{abcde}
GLK 14313	22.97 ± 1.66 ^a	24.63 ± 0.13 ^{ghij}	41.02 ± 1.95 ^c	35.62 ± 9.13 ^{bcd}	1.23 ± 0.14 ^{cd}	4.59 ± 0.23 ^d	79.64 ± 6.99 ^e	73.71 ± 4.24 ^{fg}	0.21 ± 0.07 ^{bed}	1.64 ± 0.23 ^{gh}
GLK 28127	18.22 ± 0.16 ^b	25.47 ± 0.03 ^{defg}	37.11 ± 1.95 ^{cde}	34.71 ± 9.14 ^{cdef}	2.05 ± 0.11 ^{abcd}	9.69 ± 0.61 ^{abc}	61.66 ± 5.40 ^e	101.68 ± 3.44 ^{bcd}	0.26 ± 0.05 ^{bed}	3.28 ± 0.13 ^{abcd}
GLK 07-42	15.51 ± 0.82 ^c	23.15 ± 0.1 ^m	39.06 ± 1.77 ^{cd}	32.42 ± 21.11 ^{ghij}	0.96 ± 0.30 ^d	5.49 ± 0.23 ^d	122.80 ± 3.00 ^{bcd}	131.32 ± 5.85 ^{abcde}	0.43 ± 0.08 ^{bed}	1.31 ± 0.16 ^h
GLK 08-134	18.12 ± 0.39 ^b	25.97 ± 0.16 ^{cd}	48.83 ± 1.95 ^b	27.86 ± 4.57 ^m	2.47 ± 0.20 ^{abcd}	5.59 ± 0.63 ^{bed}	64.63 ± 5.19 ^e	84.13 ± 1.79 ^{fg}	0.26 ± 0.08 ^{bed}	1.71 ± 0.12 ^{gh}
Mean	18.00 ± 0.66	24.67 ± 0.13	43.75 ± 1.91	33.43 ± 5.17	2.15 ± 0.23	7.05 ± 0.53	77.03 ± 4.90	104.62 ± 4.42	0.31 ± 0.07	2.16 ± 0.17
IC 525199	0.87 ± 0.02 ⁱ	24.32 ± 0.19 ^{hijk}	39.06 ± 1.27 ^{cd}	30.14 ± 9.14 ^{kl}	1.46 ± 0.11 ^{cd}	9.15 ± 0.25 ^{abc}	238.73 ± 7.29 ^{abc}	167.91 ± 11.01 ^{abcde}	0.42 ± 0.15 ^{bed}	3.16 ± 0.22 ^{abcde}
IC 525202	0.84 ± 0.02 ⁱ	23.38 ± 0.13 ^{lm}	35.16 ± 1.75 ^{def}	31.97 ± 0.10 ^{hijk}	1.48 ± 0.21 ^{cd}	8.66 ± 0.23 ^{abcd}	257.63 ± 21.54 ^a	118.19 ± 10.84 ^{abcde}	0.41 ± 0.03 ^{bed}	3.31 ± 0.19 ^{abcd}
IC 525691	0.77 ± 0.03 ⁱ	23.85 ± 0.41 ^{ijklm}	35.15 ± 1.63 ^{def}	28.31 ± 9.14 ^{lm}	1.55 ± 0.18 ^{cd}	9.39 ± 0.18 ^{abc}	250.83 ± 16.15 ^{abc}	153.33 ± 6.65 ^{abcde}	0.47 ± 0.07 ^{bc}	2.72 ± 0.06 ^{bcd}
C. jud 95	0.86 ± 0.03 ⁱ	24.75 ± 0.11 ^{ghi}	29.30 ± 1.95 ^{gh}	27.40 ± 1.20 ^m	1.51 ± 0.12 ^{cd}	9.45 ± 0.34 ^{abc}	217.52 ± 20.65 ^{abcd}	93.26 ± 3.81 ^{defg}	1.07 ± 0.28 ^a	4.07 ± 0.34 ^a
C. jud 185	0.91 ± 0.02 ⁱ	25.82 ± 0.38 ^{de}	39.06 ± 1.76 ^{cd}	27.86 ± 4.57 ^m	1.41 ± 0.06 ^{cd}	7.65 ± 0.17 ^{abcd}	238.57 ± 10.00 ^{abc}	187.86 ± 8.32 ^a	0.28 ± 0.01 ^{bed}	2.86 ± 0.02 ^{abcde}
C. jud 185 B	0.87 ± 0.08 ⁱ	24.32 ± 0.19 ^{hijk}	27.34 ± 1.95 ^b	28.31 ± 9.14 ^{lm}	3.18 ± 0.15 ^{abc}	8.98 ± 0.37 ^{abc}	237.96 ± 3.18 ^{abc}	174.19 ± 4.57 ^{abcd}	0.54 ± 0.32 ^b	3.01 ± 0.06 ^{abcde}
C. jud 17148	0.76 ± 0.03 ⁱ	23.78 ± 0.03 ^{klm}	29.30 ± 1.95 ^{gh}	27.40 ± 1.00 ^m	1.70 ± 0.01 ^{cd}	6.97 ± 0.08 ^{abcd}	256.53 ± 9.49 ^{ab}	180.06 ± 4.61 ^{ab}	0.31 ± 0.00 ^{bed}	2.96 ± 0.04 ^{abcde}
C. jud 17150	0.88 ± 0.01 ⁱ	23.03 ± 0.59 ^{efgh}	35.15 ± 1.95 ^{def}	30.14 ± 9.14 ^{kl}	1.81 ± 0.13 ^{bed}	8.45 ± 0.46 ^{abcd}	259.58 ± 5.66 ^a	137.22 ± 7.71 ^{abcde}	0.36 ± 0.05 ^{bed}	2.87 ± 0.16 ^{abcde}
C. jud ILWC	1.30 ± 0.05 ⁱ	24.22 ± 0.28 ^{hijkl}	39.06 ± 1.66 ^{cd}	33.34 ± 13.7 ^{ghi}	2.07 ± 0.17 ^{abcd}	8.39 ± 0.58 ^{abcd}	240.80 ± 12.06 ^{abc}	179.46 ± 12.51 ^{abc}	0.25 ± 0.03 ^{bed}	3.56 ± 0.28 ^{abc}
30										
C. pin ILWC	0.99 ± 0.05 ⁱ	23.76 ± 0.13 ^{ijklm}	27.34 ± 1.6 ^h	30.14 ± 0.09 ^{kl}	2.65 ± 0.19 ^{abcd}	9.77 ± 0.08 ^{ab}	260.95 ± 3.44 ^a	111.03 ± 3.52 ^{abcde}	0.27 ± 0.04 ^{bed}	2.96 ± 0.15 ^{abcde}
C. pin ILWC	1.59 ± 0.04 ⁱ	26.82 ± 0.32 ^{bc}	39.06 ± 1.88 ^{cd}	27.40 ± 0.09 ^m	2.53 ± 0.15 ^{abcd}	7.79 ± 0.38 ^{abcd}	150.49 ± 10.90 ^{abcde}	99.00 ± 3.78 ^{cde}	0.44 ± 0.02 ^{bed}	3.69 ± 0.18 ^{ab}
261										
EC 366338	0.84 ± 0.02 ⁱ	23.82 ± 0.13 ^{ijklm}	31.25 ± 1.95 ^{gh}	26.49 ± 9.14 ^m	1.91 ± 0.10 ^{bed}	8.63 ± 0.26 ^{abcd}	224.02 ± 22.38 ^{abcd}	119.72 ± 13.08 ^{abcde}	0.36 ± 0.04 ^{bed}	3.30 ± 0.39 ^{abcd}
EC 366342	0.84 ± 0.01 ⁱ	24.78 ± 0.28 ^{efgh}	33.20 ± 1.95 ^{efg}	27.40 ± 18.27 ^m	1.84 ± 0.05 ^{bed}	7.80 ± 0.19 ^{abcd}	120.31 ± 10.47 ^{cde}	90.65 ± 3.37 ^{efg}	0.40 ± 0.01 ^{bed}	3.29 ± 0.25 ^{abcd}
EC 556270 R	0.81 ± 0.03 ⁱ	23.38 ± 0.07 ^{hijk}	31.25 ± 1.8 ^{gh}	27.86 ± 4.57 ^m	1.43 ± 0.17 ^{cd}	8.58 ± 0.66 ^{abcd}	141.39 ± 11.89 ^{abcde}	85.42 ± 7.62 ^{fg}	0.20 ± 0.05 ^{cd}	2.94 ± 0.27 ^{abcde}
C. ech 17159	0.83 ± 0.03 ⁱ	24.53 ± 0.03 ^{hijk}	27.34 ± 1.75 ^b	27.40 ± 1.75 ^m	1.56 ± 0.21 ^{cd}	9.24 ± 0.29 ^{abc}	147.33 ± 12.07 ^{abcde}	89.36 ± 6.12 ^{efg}	0.27 ± 0.02 ^{bed}	2.70 ± 0.19 ^{bcd}
Mean	0.93 ± 0.03	24.30 ± 0.22	33.20 ± 1.79	28.77 ± 5.23	1.87 ± 0.13	8.59 ± 0.30	216.18 ± 9.81	132.44 ± 7.17	0.40 ± 0.07	3.67 ± 0.19

Data represent the mean ± SD of triplicates and values with different letters in the same column are significantly different ($p < 0.05$)

method. Crude protein content higher than 26.35% is categorized under higher crude protein group, value between 24.69 and 26.35% is placed under medium crude protein content and lower than 24.69% is placed under low crude protein group ($H \geq 26.35 \leq 24.69$). Three genotypes namely GL 12021 (Desi), GNG 2171 (Desi) and C. pin ILWC 261 (Wild) possessed higher protein content in the range of 26.35 to 28.00%. Ten genotypes possessed medium range of crude protein content ranging from 24.69 to 26.35%. Seventeen genotypes had lower crude protein content ranging from 23.03 to 24.69%. Differences of crude protein content between desi and kabuli types have been reported to be inconsistent. Our results are in accordance with Gaur et al. (2008) who reported that crude protein content in desi chickpea (29.2%) was found to be higher than kabuli chickpea (20.5%). However Ghribi et al. (2015) reported that crude protein content in desi and kabuli chickpea cultivars differ significantly as desi chickpea genotypes possessed lower amount of crude protein (20.29%) and kabuli possessed the higher amount of crude protein (24.51%). Singh et al. (2010) reported that protein content in chickpea genotypes ranged between 15.7 and 31.5%.

The total carbohydrates in chickpea seeds are up to 62% and the major proportion is of starch. One distinguish feature of legumes is that legume carbohydrates are digested slowly and due to this reason legumes are considered as low glycemic index foods that prevent diabetes, heart disease and obesity (Jenkins et al. 2012). Total soluble sugar content in chickpea genotypes varied from 27.34 to 54.68 mg/g with mean value of 36.59 mg/g (Table 1). Among cultivated genotypes, desi genotypes possessed total soluble sugar content in the range of 27.34 to 54.68 mg/g with mean value of 38.08 mg/g and kabuli genotypes possessed total soluble sugar content in the range of 37.11 to 52.73 mg/g with an average value of 43.75 mg/g. Sugar content in wild species ranged from 27.34 to 39.06 mg/g with mean value of 33.20 mg/g. Maheri-Sis et al. (2008) reported that total soluble sugar content in kabuli genotypes was higher as compared to desi genotypes.

Total starch content in chickpea genotypes ranged from 26.49 to 39.27% with mean value of 31.43% (Table 1). Wild species (EC 366338) had the lowest starch content and desi genotype (GL 13042) had the highest starch content. In desi genotypes, starch content varied from 30.60 to 39.27% and in kabuli genotypes it ranged from 27.86 to 36.53%. Wild species possessed total starch content in the range of 26.49 to 33.34% with mean value of 28.77%. Six genotypes comprising four desi GL 12021, GL 13042, GL 14015, GL 15003 and two kabuli L 552, GLK 14313 possessed higher total starch content that ranged from 35.62 to 39.27%. Nine genotypes possessed medium

range of starch content from 30.75 to 35.01% and fifteen genotypes had lower starch content in the range of 26.49 to 30.60%. Starch content among desi and kabuli genotypes varied significantly from 30.2 to 55.2% and 38.3 to 51.3% respectively (Wang et al. 2017). Amylose content of starches from kabuli and desi chickpea types ranged from 43.24 to 47.22 and 28.26 to 52.82%, respectively (Singh et al. 2010). Sekhon et al. (2017) reported that starch content in pigeonpea cultivars ranged from 27.27 to 52.12% and among wild species it ranged from 9.04 to 19.12%.

Mineral elements are involved in all defense mechanisms as they are important components of cells and enzymes. They also act as inhibitors, activators and regulators of metabolism. Chickpea can overcome mineral malnutrition as it is a good source of iron and zinc. As compared to other pulses, manganese, zinc and phosphorous are present in higher amount in chickpea (Raes et al. 2014). Zinc content in chickpea genotypes varied from 0.96 mg/100 g to 4.05 mg/100 g (Table 1). Content of iron in chickpea genotypes ranged from 4.56 mg/100 g to 9.87 mg/100 g (Table 1). Zinc content in desi, kabuli and wild species ranged from 1.63 to 3.76; 0.96 to 4.05; 1.41 to 3.18 mg/100 g and the corresponding iron content ranged from 4.56 to 9.83; 4.59 to 9.87 and 6.97 to 9.77 mg/100 g respectively. Thavarajah and Thavarajah (2012) reported that Zn and Fe content in chickpea cultivars ranged from 3.7 mg/100 g to 7.4 mg/100 g and 4.6 mg/100 g to 6.7 mg/100 g.

Calcium content in chickpea genotypes ranged from 40.83 mg/100 g to 260.95 mg/100 g (Table 1). Magnesium content ranged from 71.96 to 187.86 mg/100 g (Table 1). Copper content in chickpea genotypes was found in the range of 0.13 mg/100 g to 1.07 mg/100 g (Table 1). Manganese content ranged from 1.31 mg/100 g to 3.69 mg/100 g (Table 1). Calcium, magnesium, copper and manganese content in desi, kabuli and wild genotypes ranged from 40.83 to 246.60, 56.44 to 122.80, 217.52 to 260.95 and 1.96 to 145.12 mg/100 g; 73.71 to 132.27, 85.42 to 187.86, 0.13 to 0.40 and 0.21 to 0.43 mg/100 g and 0.20 to 1.07 and 1.80 to 3.49, 1.31 to 3.28 and 2.70 to 4.07 mg/100 g. Wild species had higher calcium, magnesium and manganese content as compared to cultivated genotypes. Thavarajah and Thavarajah (2012) reported that calcium content ranged from 93 to 197 mg/100 g and magnesium content ranged from 125 to 159 mg/100 g in chickpea cultivars. Khan et al. (2015) reported that copper and manganese content of twenty-eight chickpea genotypes ranged from (0.66 to 1.04 mg/100 g) and (1.3 to 2.1 mg/100 g). Parmar et al. (2017) reported that hard to cook kidney bean grains had significantly higher Ca and Zn while Cu, Mn and Fe were lower than easy to cook kidney bean grains. They found that most of the minerals were

correlated with the colour of the grains as hard to cook grains from light coloured accessions had lower Zn content.

Bound fructose of sucrose and raffinose series oligosaccharides

Raffinose oligosaccharides (RFO's) are galactosyl derivative of sucrose and characterized by the presence of α (1 $\rightarrow$ 6) linkage between the galactose residue and the C-6 of the glucose moiety of sucrose (Gangola et al. 2013). The highest content of raffinose oligosaccharides was found in wild species (EC 366342) 10.33 mg/g and the lowest content was found in kabuli genotypes (GLK 14313) 0.66 mg/g (Table 2). In kabuli genotypes, bound fructose content ranged from 3.66 to 4.33 mg/g except in GLK 08-134 which has the lowest bound fructose content (0.66 mg/g). Bound fructose content in desi chickpea varieties found to be in range of 2.0 to 5.33 mg/g with the mean value of 3.80 mg/g. In wild chickpea species it ranged from 5.0 to 10.33 mg/g with an average value of 7.73 mg/g. Eight genotypes from wild species had higher bound fructose content (8.33 to 10.33 mg/g).

Tannins

Tannins constitute low molecular weight (0.5–3 kDa) naturally occurring anti-nutritional compounds. They precipitate with proteins and form complexes with proline rich proteins, polysaccharides and alkaloids, some of these are water soluble while some are insoluble. Binding of tannin with enzyme proteins or minerals cause inactivation of digestive enzymes and reduced protein digestion. Tannin content in chickpea genotypes ranged from 9.08 mg/g (GL 14015) to 18.47 mg/g (C. jud ILWC 30) with mean value of 13.94 mg/g (Table 2). The average tannin content in desi, kabuli and wild species was found to be 11.93, 10.63 and 16.38 mg/g respectively. Genotypes with tannin content higher than 15.34 mg/g are high tannin group; between 12.21 and 15.34 mg/g are medium tannin group and lower than 12.21 mg/g is placed under low tannin group ($H \geq 15.34 \leq 12.21$). Kaur et al. (2014) reported that tannin content in chickpea seeds ranged from 5.44 to 10.87 mg/g. Bulbula and Urga (2018) reported that tannin content in chickpea seeds was found to be 17.52 mg/g.

Phytic acid

Phytic acid (myo-inositol hexaphosphate) is the main inhibitor of iron and zinc absorption and form protein complexes. In gastrointestinal tract, absorption of minerals like calcium, copper, magnesium, iron and zinc are adversely affected by formation of complexes between

minerals and phytic acid (Tiwari and Singh 2012). Due to the lack of sufficient level of phytate degrading enzyme activity in monogastric animals, metabolization of phytic acid is impossible and excreted in manure. Kabuli genotypes had phytic acid content in medium range i.e. from 9.43 to 13.67 mg/g (Table 2) except in GLK 08-134 which had higher content of phytic acid (17.92 mg/g). Phytic acid content in desi genotypes ranged from 8.48 to 18.39 mg/g with an average value of 12.92 mg/g. All wild species had lower phytic acid content that ranged from 4.24 to 8.48 mg/g. The chickpea genotypes were categorized into high, low and medium groups on the basis of phytic acid content ($H \geq 13.68 \leq 8.96$). Mondor et al. (2009) reported that phytic acid content ranged from 3.49 to 11.52 mg/g in desi genotypes and it ranged from 3.45 to 12.35 mg/g in kabuli genotypes. Vadivel and Biesalski (2010) reported that phytic acid content in wild type legume seeds was found to be 0.98 to 3.14 g/100 g. In our present investigation phytic acid content in desi genotypes was found to be higher than kabuli genotypes and wild species had phytic acid content in lower range. Shi et al. (2018) reported that phytic acid content in whole chickpea, split chickpea and desi chickpea was found to be 11.33 mg/g, 11.53 mg/g and 14.00 mg/g respectively.

Trypsin inhibitor activity

Protease inhibitor interfere with digestion by irreversibly binding to digestive enzymes trypsin and chymotrypsin and also due to their resistance to pepsin and acidic pH of human digestive tract. Presence of trypsin inhibitor in human foods led to impaired growth, pancreatic hyperplasia and metabolic disturbance of sulphur and amino acid utilization. The trypsin inhibitor content in chickpea genotypes varied significantly from 8.23 to 150.18 IU/g (Table 2). Trypsin inhibitor content in desi genotypes ranged from 32.91 to 112.32 IU/g with mean value of 80.08 IU/g. Trypsin inhibitor in kabuli genotypes ranged from 38.53 to 64.47 IU/g with mean value of 47.83 IU/g. All the wild species had higher trypsin inhibitor content ranging from 122.73 to 150.18 IU/g except in C. jud ILWC 30 and C. pin ILWC 261. Genotypes with trypsin inhibitor content higher than 102.87 IU/g are categorized under high trypsin inhibitor content group; values between 55.55 and 102.87 IU/g are medium group and lower than 55.55 IU/g are low trypsin inhibitor group ($H \geq 102.87 \leq 55.55$). Gupta et al. (2017) reported that trypsin inhibitor content in chickpea genotypes ranged from 111.5 to 218.4 TIU/g.

Saponins

Saponins are naturally occurring surface active glycosides that reduce absorption of nutrients and cause systemic

Table 2 Distribution of total phenol (mg/100 g), flavonol (mg/100 g), bound fructose (mg/g), tannin (mg/g), phytic acid (mg/g), tryptsin inhibitor (IU/g) and saponin (mg/g) content in cultivated and wild chickpea genotypes

Genotypes	Total Phenol	Flavonol	Bound fructose	Tannin	Phytic acid	Tryptsin inhibitor	Saponin
GL 12021	83.52 ± 2.12 ^{fg}	12.67 ± 0.92 ^{efg}	4.33 ± 0.33 ^{ghij}	13.28 ± 0.15 ^{ijk}	8.48 ± 0.95 ^{fghi}	72.37 ± 0.25 ^k	6.49 ± 0.11 ^{ij}
GL 13037	62.02 ± 0.71 ^{ijk}	9.77 ± 0.61 ^{ijkl}	3.33 ± 0.02 ^{ijk}	11.75 ± 0.30 ^{klmn}	9.90 ± 0.47 ^f	32.91 ± 1.76 ^o	6.54 ± 0.05 ^{hij}
GL 13042	56.56 ± 5.11 ^{ijkl}	13.18 ± 1.34 ^e	4.66 ± 1.33 ^{ghi}	13.96 ± 0.38 ^{ghi}	12.73 ± 0.42 ^e	50.45 ± 5.04 ^{mn}	6.70 ± 0.22 ^{ghij}
GL 14015	55.85 ± 1.94 ^{ijkl}	12.88 ± 0.65 ^{ef}	3.99 ± 0.66 ^{ghi}	9.08 ± 0.07 ^p	15.56 ± 0.47 ^{bcd}	98.72 ± 4.28 ^{hi}	7.25 ± 0.11 ^{fghi}
GL 15003	48.28 ± 2.47 ^{lm}	6.39 ± 0.27 ^{no}	2.66 ± 0.07 ^{jk}	11.98 ± 0.53 ^{iklm}	18.39 ± 0.47 ^a	89.35 ± 2.55 ^{ij}	5.51 ± 0.11 ^l
GL 15017	38.59 ± 4.06 ^{mno}	5.04 ± 0.15 ^o	2.02 ± 0.09 ^{kl}	10.61 ± 0.07 ^{mno}	13.20 ± 0.95 ^{de}	61.53 ± 1.34 ^{klm}	8.97 ± 0.11 ^{abcd}
GL 29078	42.46 ± 4.41 ^{mn}	10.74 ± 0.19 ^{hij}	3.66 ± 0.33 ^{hijk}	13.43 ± 0.15 ^{hij}	15.09 ± 0.94 ^{cde}	91.08 ± 8.41 ^{ij}	7.51 ± 0.05 ^{fg}
GL 29095	60.44 ± 1.24 ^{jk}	9.47 ± 0.08 ^{ijklm}	5.33 ± 0.21 ^{efgh}	12.44 ± 0.07 ^{ijkl}	17.45 ± 0.47 ^{abc}	112.32 ± 3.61 ^{fg}	9.51 ± 0.22 ^{ab}
GNG 2171	60.61 ± 1.02 ^{jk}	9.13 ± 0.19 ^{iklm}	3.33 ± 0.14 ^{ijk}	11.67 ± 0.22 ^{lmn}	8.49 ± 0.48 ^{fghi}	107.03 ± 1.51 ^{gh}	7.41 ± 0.05 ^{fg}
PBG 7	53.74 ± 1.94 ^{kl}	8.40 ± 0.08 ^{lm}	4.66 ± 0.66 ^{ghi}	11.06 ± 0.53 ^{lmno}	9.90 ± 0.47 ^f	85.04 ± 1.18 ^j	6.27 ± 0.86 ^{kl}
Mean	56.21 ± 2.50	9.77 ± 0.45	3.80 ± 0.38	11.93 ± 0.25	12.92 ± 0.70	80.08 ± 1.99	7.22 ± 0.19
L 552	61.14 ± 5.46 ^{jk}	10.23 ± 0.08 ^{ijk}	4.33 ± 0.33 ^{ghij}	9.84 ± 0.22 ^{op}	12.73 ± 0.47 ^e	38.78 ± 0.59 ^{no}	5.57 ± 0.05 ^{kl}
GLK 14313	32.24 ± 2.29 ^{op}	0.80 ± 0.04 ^p	0.66 ± 0.19 ^l	10.30 ± 0.38 ^{nop}	13.67 ± 0.48 ^{de}	38.53 ± 1.85 ^o	6.38 ± 0.54 ^{jk}
GLK 28127	27.48 ± 1.06 ^p	1.37 ± 0.23 ^p	3.66 ± 0.33 ^{hijk}	11.67 ± 0.38 ^{lmn}	9.43 ± 0.94 ^{fg}	58.09 ± 3.27 ^{lm}	7.46 ± 0.11 ^{fg}
GLK 07-42	48.01 ± 1.59 ^{lm}	5.50 ± 0.99 ^o	3.99 ± 0.66 ^{hij}	10.76 ± 0.38 ^{mno}	9.90 ± 0.47 ^f	39.29 ± 1.09 ^{no}	7.84 ± 0.27 ^{ef}
GLK 08-134	33.30 ± 1.94 ^{nop}	1.22 ± 0.38 ^p	4.33 ± 0.33 ^{ghij}	10.53 ± 0.15 ^{mno}	17.92 ± 0.94 ^{ab}	64.47 ± 1.09 ^{kl}	7.82 ± 0.17 ^{ef}
Mean	40.45 ± 2.47	3.82 ± 0.34	3.40 ± 0.37	10.63 ± 0.31	12.73 ± 0.66	47.83 ± 1.58	7.02 ± 0.23
IC 525199	97.44 ± 4.05 ^{cd}	11.04 ± 0.42 ^{bij}	5.99 ± 0.66 ^{efg}	14.96 ± 1.98 ^{egh}	5.65 ± 0.95 ^{jk}	127.01 ± 2.35 ^{de}	7.24 ± 0.32 ^{fghi}
IC 525202	98.50 ± 0.53 ^{cd}	16.26 ± 0.92 ^d	6.66 ± 0.05 ^{def}	17.40 ± 0.30 ^{abcd}	6.13 ± 0.47 ^{ijk}	138.94 ± 2.85 ^{abc}	7.35 ± 0.22 ^{fgh}
IC 525691	104.49 ± 1.59 ^{abc}	24.20 ± 1.45 ^a	5.02 ± 1 ^{fghi}	16.41 ± 0.07 ^{bcd}	6.6 ± 0.47 ^{hijk}	135.91 ± 3.86 ^{bcd}	8.38 ± 0.27 ^{de}
C. jud 95	84.22 ± 0.71 ^{efg}	18.17 ± 0.53 ^c	6.65 ± 0.05 ^{def}	16.87 ± 0.99 ^{bcd}	9.90 ± 0.47 ^f	122.73 ± 2.94 ^{ef}	8.49 ± 0.16 ^{cde}
C. jud 185	93.92 ± 2.65 ^{de}	8.93 ± 0.08 ^{iklm}	6.68 ± 0.05 ^{def}	17.70 ± 0.30 ^{abc}	5.18 ± 0.48 ^{jk}	129.87 ± 1.18 ^{cde}	8.70 ± 0.05 ^{bcd}
C. jud 185 B	84.75 ± 2.65 ^{efg}	11.11 ± 0.11 ^{fghij}	6.01 ± 0.06 ^{efg}	17.93 ± 0.38 ^{ab}	5.18 ± 0.42 ^{jk}	141.03 ± 0.59 ^{abc}	9.68 ± 0.05 ^a
C. jud 17148	63.08 ± 1.41 ^{ijk}	8.59 ± 0.19 ^{klm}	9.05 ± 0.55 ^{ab}	15.72 ± 0.15 ^{ef}	4.24 ± 0.47 ^k	139.86 ± 1.09 ^{abc}	8.81 ± 0.16 ^{bcd}
C. jud 17150	71.36 ± 0.18 ^{hi}	7.94 ± 0.31 ^{mn}	6.99 ± 0.33 ^{cde}	15.72 ± 0.30 ^{ef}	8.96 ± 0.47 ^{fgh}	150.18 ± 2.35 ^a	8.92 ± 0.27 ^{abcd}
C. jud ILWC 30	109.07 ± 4.05 ^{ab}	20.31 ± 0.53 ^b	9.33 ± 0.66 ^{ab}	18.47 ± 0.15 ^a	8.48 ± 0.45 ^{fghi}	15.95 ± 0.59 ^p	9.24 ± 0.05 ^{abc}
C. pin ILWC 0	79.47 ± 4.76 ^{gh}	9.85 ± 0.31 ^{ijkl}	8.33 ± 1.66 ^{bcd}	16.10 ± 0.22 ^{def}	5.66 ± 0.95 ^{jk}	137.34 ± 0.42 ^{bcd}	6.54 ± 0.05 ^{hij}
C. pin ILWC 261	63.96 ± 1.94 ^{ij}	12.75 ± 0.69 ^{efg}	8.33 ± 0.33 ^{bcd}	16.41 ± 0.07 ^{bcd}	8.48 ± 0.95 ^{fghi}	8.23 ± 0.59 ^p	8.76 ± 0.11 ^{bcd}
EC 366338	93.03 ± 4.23 ^{def}	12.06 ± 0.23 ^{efgh}	8.66 ± 0.66 ^{abc}	15.49 ± 0.07 ^{efg}	6.13 ± 0.47 ^{ijk}	142.38 ± 12.17 ^{ab}	7.84 ± 0.27 ^{ef}
EC 366342	102.73 ± 6.52 ^{bcd}	10.31 ± 0.46 ^{hijk}	10.33 ± 0.33 ^a	15.19 ± 0.07 ^{fg}	9.90 ± 1.42 ^f	131.13 ± 3.78 ^{bcd}	9.30 ± 0.11 ^{abc}
EC 556270 R	113.30 ± 2.64 ^a	10.36 ± 0.38 ^{bijk}	8.33 ± 0.33 ^{bcd}	16.33 ± 0.30 ^{cdef}	7.07 ± 1.42 ^{fghi}	137.26 ± 0.34 ^{bcd}	9.46 ± 0.27 ^{ab}
C. ech 17159	76.82 ± 0.71 ^{gh}	8.97 ± 0.19 ^{iklm}	9.66 ± 0.33 ^{ab}	14.96 ± 0.15 ^{fgh}	5.65 ± 0.95 ^{kj}	126.43 ± 0.08 ^{de}	9.08 ± 0.43 ^{abcd}
Mean	89.07 ± 2.57	12.72 ± 0.45	7.73 ± 0.47	16.38 ± 0.37	6.88 ± 0.81	118.095 ± 1.68	8.52 ± 0.19

Data represent the mean ± SD of triplicates and values with different letters in the same column are significantly different ($p < 0.05$). One inhibitor unit is the quantity of inhibitor that inhibits 50% of bovine trypsin activity

toxicity by directly binding and inactivating enzymes. Saponins causes leakage of cells by creating holes, disturb the fluidity and causes lysis of red blood cells. Saponin affects metabolism in different ways like reduced cholesterol, depressed growth rate, erythrocyte haemolysis and reduced nutrient absorption. The average content of saponin in desi and kabuli genotypes was found to be 7.22 and 7.02 mg/g, respectively (Table 2). However all the wild species had higher saponin content that ranged from 8.38 to 9.68 mg/g except IC 525199, IC 525202 and EC 366338 which had medium saponin content in the range of 7.24 to 7.84 mg/g and *C. pin* ILWC 0 had lower saponin content (6.54 mg/g). Choudhary et al. (2015) reported that saponin content in kabuli chickpea genotypes varied from 4.98 to 12.23 mg/g. Sekhon et al. (2017) reported that saponin content ranged from 4.73 to 17.98 mg/g in pigeonpea genotypes.

Total phenol and flavonol in chickpea genotypes

Phenols bind to positively charged proteins, amino acids or multivalent cations and minerals such as iron, zinc and calcium in foods and decrease their bioavailability. On the other hand prevention of reactive oxygen species and removal of existing reactive oxygen species from blood are beneficial effect of phenols. Singh et al. (2017) reported that primary phenolic compounds present in legume seeds are phenolic acids, flavonoids and condensed tannins which play significant roles in many physiological and metabolic processes. These phenolic compounds had numerous health benefits as they act as anticarcinogenic, anti-thrombotic, anti-ulcer, anti-atherogenic, anti-allergenic, anti-inflammatory, antioxidant, immunomodulating, anti-microbial, cardioprotective and analgesic agents. Seed coat of legumes has high concentration of phenolic acids and acts as protective barrier for the cotyledon. The total phenol content in chickpea genotypes lied in the range of 27.48 mg/100 g to 113.30 mg/100 g (Table 2). All kabuli genotypes have lower total phenol content that ranged from 27.48 mg/100 g to 48.01 mg/100 g except in L 552 which has medium range of total phenol content. Wild species possessed total phenol content in higher and medium range that ranged from 63.08 mg/100 g to 113.30 mg/100 g with an average value of 89.08 mg/100 g. Total phenol content varied from 38.59 mg/100 g to 83.52 mg/100 g in desi genotypes. Higher total phenolic content in desi and wild genotypes in comparison to kabuli genotypes might be due to their dark coloured seed coat. Xu et al. (2007) compared different legumes for total phenol content and reported that soybeans (1.57–5.57 mg GAE/g), chickpeas (0.98 mgGAE/g), yellow peas (0.85–1.14 mg GAE/g) and green peas (0.65–0.99 mg GAE/g) have lower total phenol content than lentils (4.86–9.60 mg GAE/g). Sekhon et al. (2017)

studied reported that total phenol content in pigeonpea wild species *C. Scarabaeoides* ranged from 69 mg/100 g to 129 mg/100 g. Flavonol content in chickpea genotypes ranged from 0.80 mg/100 g (GLK 14313) to 24.20 mg/100 g (IC 525691) with an average value of 10.25 mg/100 g (Table 2). Kabuli genotypes possessed lower flavonol content ranging from 0.08 mg/100 g to 0.55 mg/100 g. Flavonol content in desi genotypes varied from 5.04 mg/100 g to 13.18 mg/100 g. Flavonol content varied from 7.94 mg/100 g to 24.20 mg/100 g in wild species with an average value of 12.72 mg/100 g. Sekhon et al. (2017) reported that flavonol content in pigeonpea genotypes ranged from 5.32 mg/100 g to 16.02 mg/100 g. In present investigation, the higher total phenol and flavonol content in desi and wild species as compared to kabuli cultivars could be due to their dark coloured seeds as dark coloured legume had higher total phenol content as compared to light coloured seeds.

Free radical scavenging activity (DPPH)

DPPH is a stable free radical having maximum absorbance at 517 nm in methanol. It is used to determine antioxidant activity in natural compounds and its assay is mainly based on an electron transfer reaction and hydrogen-atom abstraction. DPPH free radical scavenging activity in chickpea genotypes ranged from 10.57 to 89.61% with mean value of 56.72% (Table 3). All kabuli genotypes possessed DPPH activity in the lower range (10.57 to 25.64%) and all wild species possessed DPPH activity in higher range (64.74 to 89.61%) except *C. jud* 17148, *C. jud* 17150 and *C. pin* ILWC 0 which had DPPH activity in medium range. DPPH scavenging activity in desi genotypes ranged from 21.02 to 75.57% with an average value of 49.40%. DPPH scavenging activity in wild chickpea species was higher than desi and kabuli genotypes indicating their higher antioxidative potential which might help to reduce oxidative stress in them. Gupta et al. (2017) reported that DPPH radical scavenging activity in forty chickpea genotypes ranged from 32.6 to 58.9%.

Ferric reducing antioxidant power (FRAP)

The reaction measures reduction of ferric-2,4,6-tripyridyl-triozine (TPTZ) complex to ferrous form and an intense blue colour with an absorption maximum at 593 nm develops. FRAP is a reasonable screening method for the ability to maintain redox status in cells or tissues because reaction detects compounds with redox potentials of < 0.7 V. FRAP activity in chickpea genotypes varied from 0.52 mg/g (GLK 08-134) to 2.74 mg/g (*C. pin* ILWC 0) (Table 3). All the kabuli genotypes had lower FRAP activity and all desi genotypes had medium FRAP activity

Table 3 Distribution of DPPH radical scavenging activity (%), FRAP (mg/g), reducing power (mg/g), hydroxyl radical scavenging activity (%), superoxide anion radical scavenging activity (%) and nitric oxide radical scavenging activity (%)

Genotypes	DPPH	FRAP	Reducing Power	Hydroxyl radical scavenging power	Superoxide anion radical scavenging activity	Nitric oxide radical scavenging activity
GL 12021	63.97 ± 1.41 ^{efg}	1.71 ± 0.12 ^{efg}	33.09 ± 1.34 ^{fg}	28.89 ± 0.47 ^{ab}	35.91 ± 5.26 ^{cde}	61.18 ± 0.93 ^{bcd}
GL 13037	46.28 ± 0.09 ^{kl}	1.56 ± 0.10 ^g	26.13 ± 0.82 ^{jk}	3.63 ± 3.15 ^{kl}	48.43 ± 0.50 ^a	64.80 ± 0.41 ^{ab}
GL 13042	46.02 ± 2.69 ^{kl}	1.97 ± 0.10 ^{def}	30.21 ± 0.17 ^h	2.77 ± 1.33 ^l	40.67 ± 2.00 ^{bcd}	58.49 ± 0.31 ^{de}
GL 14015	52.82 ± 3.20 ^{ij}	1.66 ± 0.27 ^{fg}	32.36 ± 0.61 ^g	7.65 ± 2.00 ^{ikl}	37.04 ± 0.87 ^{cde}	56.25 ± 0.41 ^{ef}
GL 15003	48.07 ± 2.05 ^{jk}	1.49 ± 0.19 ^g	25.06 ± 0.26 ^{jk}	15.02 ± 1.72 ^{fghi}	25.03 ± 0.12 ^f	61.28 ± 1.86 ^{bcd}
GL 15017	21.02 ± 3.07 ^{no}	1.39 ± 0.01 ^{ghi}	23.00 ± 0.17 ^{lm}	7.94 ± 1.14 ^{ikl}	41.17 ± 3.75 ^{abcd}	62.94 ± 2.07 ^{abcd}
GL 29078	39.03 ± 0.70 ^m	1.40 ± 0.22 ^{gh}	24.59 ± 0.47 ^{kl}	9.76 ± 8.51 ^{ijkl}	48.06 ± 0.87 ^{ab}	53.00 ± 0.62 ^{fg}
GL 29095	42.37 ± 0.32 ^{lm}	1.55 ± 0.12 ^g	25.32 ± 0.60 ^{jk}	12.05 ± 2.00 ^{ghij}	32.79 ± 0.37 ^e	65.22 ± 1.45 ^{ab}
GNG 2171	75.57 ± 0.70 ^d	0.92 ± 0.03 ^{jk}	24.63 ± 0.17 ^{kl}	21.62 ± 0.09 ^{cdef}	42.67 ± 1.25 ^{abc}	61.59 ± 0.72 ^{bcd}
PBG 7	58.78 ± 0.32 ^{gm}	0.86 ± 0.02 ^{ikl}	22.66 ± 0.35 ^{lm}	6.41 ± 1.14 ^{ikl}	12.64 ± 0.25 ^{gh}	58.80 ± 1.04 ^{cde}
Mean	49.40 ± 1.63	1.46 ± 0.12	26.70 ± 0.50	11.58 ± 2.16	36.45 ± 1.39	60.38 ± 0.98
L 552	24.93 ± 1.73 ⁿ	0.74 ± 0.01 ^{kl}	16.09 ± 0.64 ^o	12.24 ± 2.00 ^{ghij}	17.07 ± 0.06 ^g	63.25 ± 0.52 ^{abc}
GLK 14313	13.52 ± 2.11 ^p	1.09 ± 0.11 ^{hij}	15.62 ± 0.17 ^o	13.30 ± 0.19 ^{ghij}	25.28 ± 0.87 ^f	67.18 ± 1.55 ^a
GLK 28127	10.57 ± 1.60 ^p	0.94 ± 0.05 ^{jk}	17.25 ± 0.52 ^o	10.62 ± 4.21 ^{hijk}	40.67 ± 0.25 ^{bcd}	59.42 ± 0.41 ^{cde}
GLK 07-42	25.64 ± 0.76 ⁿ	1.06 ± 0.04 ^{ijk}	19.48 ± 0.43 ⁿ	12.34 ± 1.14 ^{ghij}	39.29 ± 0.62 ^{cde}	56.52 ± 2.07 ^{ef}
GLK 08-134	19.55 ± 2.24 ^o	0.52 ± 0.08 ^l	22.18 ± 0.91 ^m	11.67 ± 0.47 ^{ghij}	34.79 ± 0.87 ^{de}	61.59 ± 0.93 ^{bcd}
Mean	18.85 ± 1.69	0.88 ± 0.06	18.12 ± 0.53	12.04 ± 1.61	31.43 ± 0.54	61.59 ± 1.10
IC 525199	89.61 ± 0.51 ^a	2.26 ± 0.05 ^{cd}	35.87 ± 0.52 ^{de}	15.31 ± 2.58 ^{fghi}	11.51 ± 1.37 ^{gh}	49.07 ± 4.35 ^{gh}
IC 525202	77.62 ± 1.98 ^d	2.09 ± 0.05 ^{cd}	40.81 ± 0.56 ^b	21.53 ± 1.72 ^{cdef}	10.38 ± 0.50 ^{gh}	42.03 ± 1.24 ^{ikl}
IC 525691	84.80 ± 0.19 ^{ab}	2.41 ± 0.11 ^{abc}	34.42 ± 0.09 ^{ef}	31.29 ± 0.38 ^a	10.26 ± 2.62 ^{gh}	41.82 ± 0.41 ^{ikl}
C. jud 95	74.10 ± 0.51 ^d	2.00 ± 0.08 ^{de}	34.03 ± 0.13 ^{efg}	26.98 ± 0.28 ^{abc}	8.13 ± 2.25 ^h	49.17 ± 1.55 ^{gh}
C. jud 185	78.58 ± 0.76 ^{cd}	2.63 ± 0.08 ^{ab}	37.46 ± 1.16 ^{cd}	9.18 ± 1.62 ^{ijkl}	16.14 ± 0.50 ^g	43.37 ± 0.31 ^{ijk}
C. jud 185 B	77.75 ± 2.62 ^d	2.22 ± 0.02 ^{cd}	40.12 ± 0.22 ^b	22.77 ± 0.86 ^{bcd}	10.01 ± 0.12 ^{gh}	49.59 ± 0.93 ^{gh}
C. jud 17148	61.85 ± 0.32 ^{efgh}	2.02 ± 0.05 ^{de}	28.58 ± 0.26 ^{hi}	26.88 ± 0.19 ^{abc}	10.63 ± 1.50 ^{gh}	38.20 ± 0.31 ^{lmno}
C. jud 17150	59.48 ± 3.46 ^{fgh}	2.66 ± 0.02 ^{ab}	33.26 ± 0.22 ^{fg}	11.86 ± 0.47 ^{ghij}	10.51 ± 0.37 ^{gh}	47.10 ± 1.14 ^{hi}
C. jud ILWC 30	73.71 ± 1.02 ^d	2.11 ± 0.08 ^{cd}	38.96 ± 0.95 ^{bc}	26.02 ± 0.47 ^{abc}	10.26 ± 2.62 ^{gh}	35.61 ± 0.62 ^{no}
C. pin ILWC 0	57.17 ± 1.15 ^{hi}	2.74 ± 0.09 ^a	34.03 ± 0.39 ^{efg}	26.12 ± 0.95 ^{abc}	7.38 ± 0.25 ^h	41.30 ± 1.14 ^{ijklm}
C. pin ILWC 261	66.66 ± 1.02 ^e	1.64 ± 0.02 ^{fg}	26.73 ± 0.13 ^{ij}	24.78 ± 0.38 ^{abcd}	9.76 ± 2.62 ^{gh}	45.34 ± 2.28 ^{hij}
EC 366338	83.20 ± 0.38 ^{bc}	2.36 ± 0.13 ^{bc}	37.33 ± 1.21 ^{cd}	21.05 ± 0.66 ^{defg}	10.26 ± 0.12 ^{gh}	33.95 ± 0.21 ^o
EC 366342	75.38 ± 2.30 ^d	2.62 ± 0.10 ^{ab}	37.89 ± 0.90 ^{cd}	17.99 ± 2.00 ^{defg}	6.25 ± 0.87 ^h	36.75 ± 1.35 ^{mno}
EC 556270 R	88.78 ± 0.57 ^a	2.38 ± 0.04 ^{bc}	43.60 ± 0.69 ^a	7.84 ± 0.47 ^{ikl}	5.75 ± 0.62 ^h	39.44 ± 2.59 ^{klmn}
C. ech 17159	64.74 ± 0.76 ^{ef}	2.17 ± 0.03 ^{cd}	32.40 ± 0.82 ^{fg}	17.22 ± 1.43 ^{efgh}	7.25 ± 2.12 ^h	33.64 ± 0.52 ^o
Mean	74.23 ± 1.18	2.29 ± 0.07	35.70 ± 0.55	20.46 ± 0.97	9.64 ± 1.10	41.76 ± 1.26

Data represent the mean ± SD of triplicates and values with different letters in the same column are significantly different ($p < 0.05$)

except GNG 2171 and PBG7 which had FRAP activity in the range of (0.92 and 0.86 mg/g), respectively. All wild species possessed higher FRAP activity except *C. pin* ILWC 261 which had FRAP activity in the medium range (1.64 mg/g). Quintero-Soto et al. (2018) reported that FRAP values in desi chickpeas were found to be higher than those of kabuli chickpeas.

Hydroxyl radical scavenging activity

Hydroxyl radical is the most reactive among reactive oxygen radicals. It causes cell damage and other diseases

by readily reacting with other groups or substances in body. Hydroxyl radicals react with polypeptides, DNA (thymine and guanosine) and proteins. Scavenging of hydroxyl radical is very important for protection against various metabolic disorders. The scavenging rate of hydroxyl radical in chickpea cultivars ranged from 2.77 (GL 13042) to 31.29% (IC 525691) with an average value of 16.10% (Table 3). Kabuli genotypes have hydroxyl radical scavenging activity ranging from 10.62 to 13.30% with an average value of 12.04%. In desi genotypes, it ranged from (2.77 to 28.89%) with an average value of 11.58%. Wild species have the average value of 20.46% with the scavenging activity

ranging from 7.84 to 31.29%. Zhao et al. (2013) reported that hydroxyl radical scavenging activity in cowpea, kidney bean, chickpea and lentil was found to be 58.64%, 85.68%, 66.22% and 60.09%, respectively.

Superoxide anion radical scavenging activity

Superoxide radicals are highly toxic species which are generated by numerous biological reactions such as oxidation of haemoglobin and normal catalytic function of a number of metabolic enzymes. The concentration of superoxide free radicals increases under condition of oxidative stress and these are also generated by auto-oxidation processes or by enzymes. These free radicals are potential precursor of highly reactive species, powerful and dangerous hydroxyl radical. Superoxide anion radical scavenging activity in wild species was found to be lower (5.75 to 16.14%) as compared to desi (12.64 to 48.43%) and kabuli (17.07 to 40.67%), respectively (Table 3). Sekhon et al. (2017) investigated cultivated and wild species of pigeonpea and found that superoxide radical scavenging activity of pigeonpea ranged from 13.33 to 52.45% and in wild species it ranged from 15.5 to 24.5%.

Total reducing power

The presence of reductones is associated with reducing properties which can break the free radical chain with the donation of hydrogen atom. Reductones avert the peroxide formation by reacting with precursors of peroxides (Kumaran and Karunakaran 2007). Reducing power content in desi, kabuli and wild chickpea genotypes ranged from 22.66 to 33.09; 15.62 to 22.18 and 26.73 to 43.60 mg/100 g, respectively (Table 3).

Nitric oxide radical scavenging activity

Nitric oxide is a free radical and has an unpaired electron. Free radicals like nitrate, nitrite and s-nitrosothiols are formed when nitric oxide react rapidly in the intracellular environment. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. All kabuli genotypes possessed higher nitric oxide radical scavenging activity ranging from 59.42 to 67.18% with mean value of 61.59% (Table 3). Desi genotypes also possessed higher nitric oxide radical scavenging activity ranging from 56.52 to 65.22% except in GL29078 which had nitric oxide scavenging activity of 53.00%. In wild species, nitric oxide radical scavenging activity is in the lower range from 33.64 to 43.37% except in IC 525199, C. jud 95, C. jud 185 B, C. jud 17150 and C. pin ILWC 261 which had nitric oxide radical scavenging activity ranging from 45.34 to 49.59%. Nithiyanantham et al. (2012)

reported that raw chickpea seed extract had 54% nitric oxide radical scavenging activity and green pea had 45% nitric oxide radical scavenging activity.

Correlation analysis

Correlation analysis was carried out between all the parameters and has been represented in (Table 4) using the software SPSS. The Pearson correlation indicated the positive and negative relationships between different parameters. Bound fructose (V6) has negative correlation with seed weight ($r = -0.782$). Therefore, wild species with lower seed weight has higher bound fructose of sucrose and raffinose oligosaccharides. Tannin has significant negative correlation with seed weight ($r = -0.846^{**}$) and total sugars ($r = -0.437^{**}$). Trypsin inhibitor has negative correlation with seed weight ($r = -0.657^{**}$) and as from our results kabuli genotypes having higher seed weight had lower trypsin inhibitor content whereas wild species having lower seed weight had higher trypsin inhibitor content. Total phenol has significant and positive correlation with DPPH, FRAP, hydroxyl radical scavenging activity and reducing power. Verma et al. (2008) reported that antioxidant ability of seeds is positively correlated with phenolic content. Most of the genotypes possessing higher content of total phenol had higher level of DPPH radical scavenging activity (Tables 2, 3). Positively strong and significant correlation was observed in total phenol content and total antioxidant content in parameters FRAP, reducing power and DPPH (Yeo and Shahidi 2015). Total soluble sugar has positive correlation with total starch content ($r = 0.225^{*}$). Phytic acid has negative correlation with minerals (iron, calcium, magnesium, copper and manganese) except zinc which showed positive correlation with phytic acid. Negative correlation of minerals with phytic acid showed that as the phytic acid content increases, reduction in minerals take place because phytic acid affect the absorption of minerals (Tiwari and Singh 2012).

Conclusion

Thirty chickpea genotypes (ten desi, five kabuli and fifteen wild) compared for nutritional, antinutritional and antioxidant potential showed a lot of diversity which can be further used. In desi genotypes, GL 12021 had high crude protein content, high soluble protein content and high starch content, lower phytic acid and saponin and medium trypsin inhibitor and tannin content. GNG 2171 possessed higher crude protein content and soluble sugar content and GLK 28127 possessed crude protein and soluble sugar content in medium range whereas both possessed lower

Table 4 Correlation of biochemical parameters related to nutritional and antioxidant potential

V0	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
V1	1											
V2	NS	1										
V3	0.486**	0.403**	1									
V4	0.608**	NS	0.225*	1								
V5	− 0.782**	NS	− 0.381**	− 0.635**	1							
V6	− 0.846**	NS	− 0.437**	− 0.598**	0.723**	1						
V7	0.621**	NS	NS	0.497**	− 0.586**	− 0.672**	1					
V8	− 0.481**	NS	− 0.340**	− 0.660**	0.542**	0.543**	− 0.294**	1				
V9	− 0.657**	NS	− 0.492**	− 0.563**	0.431**	0.468**	− 0.428**	0.337**	1			
V10	− 0.809**	NS	− 0.249*	− 0.474**	0.678**	0.780**	− 0.634**	0.395**	0.505**	1		
V11	− 0.643**	NS	NS	NS	0.392**	0.587**	− 0.348**	NS	NS	0.711**	1	
V12	− 0.897**	NS	− 0.265*	− 0.542**	0.636**	0.749**	− 0.647**	0.360**	0.605**	0.881**	0.676**	1
V13	− 0.822**	− 0.304**	− 0.588**	− 0.461**	0.671**	0.799**	− 0.592**	0.446**	0.637**	0.778**	0.505**	0.715**
V14	0.709**	NS	0.412**	0.582**	− 0.728**	− 0.684**	0.569**	− 0.345**	− 0.495**	− 0.689**	− 0.381**	− 0.632**
V15	− 0.482**	NS	NS	− 0.444**	0.424**	0.539**	− 0.463**	NS	0.252*	0.456**	0.509**	0.471**
V16	− 0.841**	NS	− 0.462**	− 0.465**	0.673**	0.802**	− 0.562**	0.474**	0.616**	0.888**	0.615**	0.862**
V17	0.810**	0.339**	0.482**	0.673**	− 0.880**	− 0.785**	0.677**	− 0.527**	− 0.522**	− 0.726**	− 0.476**	− 0.694**
V18	NS	0.241*	NS	0.230*	NS	− 0.225*	0.343**	NS	NS	− 0.249*	NS	− 0.216*
V19	− 0.376**	NS	NS	NS	0.346**	0.317**	− 0.243**	NS	NS	0.386**	0.435**	0.306**
V20	− 0.681**	NS	− 0.260*	− 0.497**	0.529**	0.690**	− 0.682**	0.311**	0.500**	0.574**	0.431**	0.631**
V21	− 0.381**	NS	NS	− 0.292**	0.320* ⁸	0.442**	− 0.549**	0.215*	0.244*	0.385* ⁸	0.270**	0.409**
V22	− 0.291**	NS	NS	− 0.269*	NS	0.332**	NS	NS	NS	0.259*	0.418**	0.279**
V23	− 0.447**	NS	NS	− 0.214*	0.485**	0.536**	− 0.220**	0.281**	− 0.212*	0.367**	0.445**	0.346**
V0	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	
V1												
V2												
V3												
V4												
V5												
V6												
V7												
V8												
V9												
V10												
V11												
V12												

Table 4 continued

V0	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23
V13	1										
V14	– 0.613**	1									
V15	0.322**	– 0.491**	1								
V16	0.850**	– 0.582**	0.351**	1							
V17	– 0.758**	0.744**	– 0.467**	– 0.740**	1						
V18	– 0.294**	0.225*	NS	– 0.249*	0.331**	1					
V19	0.347**	– 0.253*	NS	0.364**	NS	NS	1				
V20	0.570**	– 0.676**	0.439**	0.552**	– 0.638**	– 0.375**	0.241**	1			
V21	0.273**	– 0.356**	0.320**	0.270**	– 0.355**	NS	NS	0.521**	1		
V22	NS	– 0.274**	0.382**	0.213*	NS	NS	0.298**	NS	NS	1	
V23	0.279**	– 0.442**	– 0.420**	0.315**	– 0.464**	NS	NS	NS	NS	NS	1

NS represent non significant coefficients. V1, Seed Weight; V2, Crude Protein; V3, Total Soluble Sugar; V4, Total Starch; V5, Bound Fructose; V6, Tannin; V7, Phytic Acid; V8, Saponin; V9, Trypsin Inhibitor; V10, Total Phenol; V11, Flavonol; V12, DPPH (2,2-diphenyl-1-picrylhydrazyl) activity; V13, Ferric Reducing Antioxidant Power; V14, Superoxide anion radical scavenging activity; V15, Hydroxyl radical scavenging activity; V16, Reducing Power; V17, Nitric Oxide radical scavenging activity; V18, Zinc; V19, Iron; V20–Calcium; V21, Magnesium; V22, Copper; V23, Manganese

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

content of tannin and phytic acid. Kabuli genotype (L 552) contained higher content of total starch, soluble sugar, Zn and Fe and lower content of tannin, saponin and trypsin inhibitor. Wild species *C. pin* ILWC 261 had higher crude protein content and lower trypsin inhibitor and phytic acid content. Kabuli genotypes possessed the lowest antioxidant activity.

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