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Research Paper

Comparative evaluation of the phenolic constituents, antioxidant properties, α-amylase and α-glucosidase inhibitory activity of field pea and cowpea cultivars

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ABSTRACT

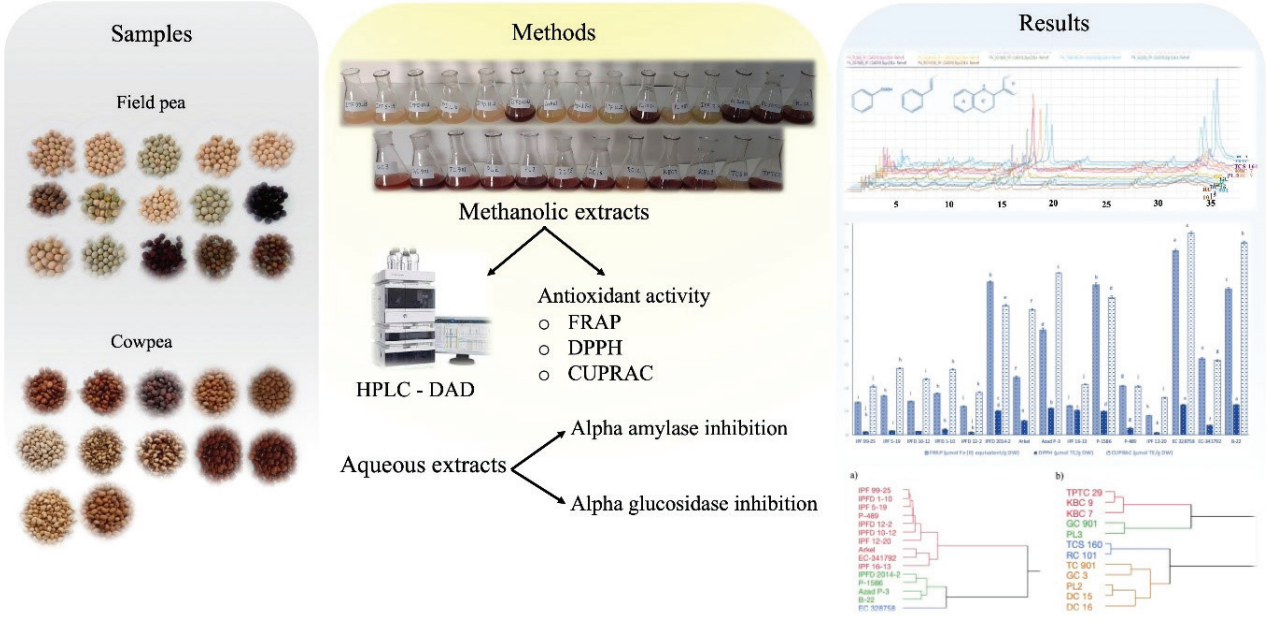
In this study, field pea and cowpea cultivars were compared for their phenolic profile, antioxidant and antidiabetic potential. Results of the present study exhibited a more diverse phenolic profile, higher antioxidant potential, as well as higher α-glucosidase inhibition potential in cowpea cultivars as compared to the field pea cultivars. Moreover, the dark-seeded cultivars showed higher phenolic content, and antioxidant activity than the light-seeded cultivars. Phenolics such as caffeic acid, ellagic acid, and quercetin were detected only in cowpeas. Overall, this study highlights the importance of these pulses as a rich source of phenolics with high antioxidant potential and explores their nutraceutical worth in the prevention and management of diabetes.

Key words: Antidiabetic potential, Antioxidant potential, Cowpea, Field pea, Phenolic profile, Pulses

INTRODUCTION

Field pea (*Pisum sativum*) and cowpea (*Vigna unguiculata*) are nutritionally important pulse crops with high genetic diversity (Muranaka *et al*. 2016, Carvalho *et al*. 2017). They are environmentally sustainable and cheap sources of protein (Rizkalla *et al*. 2002, Tharanathan and Mahadevamma 2003, Vazz Patto *et al*. 2015, Havemeier *et al*. 2017). Cowpea is an underutilized pulse crop that can be grown in arid and semi-arid parts of the world and it has

Graphical Abstract:



immense potential to ensure food and nutritional security in this era of global climate change.

Many epidemiological and interventional studies indicate that consumption of pulses reduces the risk of diseases such as cardiovascular ailments, type 2 diabetes mellitus, obesity, and colon cancer (Bazzano *et al*. 2001, Winham, Hutchins and Johnston 2007, Messina 2014). Type 2 Diabetes Mellitus (DM) has emerged as a global threat in the 21st century in developed as well as developing countries (Chatterjee *et al*. 2017, Cho *et al*. 2018). For the management of Type 2 DM, carbohydrate-metabolizing enzymes such as α amylase and intestinal α glucosidase are targeted to control postprandial hyperglycemia. Plant phenolics from diverse sources have been investigated for inhibition of α-amylase and α-glucosidase (Tresserra-Rimbau *et al*. 2014, Hanhineva *et al*. 2010, Zhang *et al*. 2015). Surprisingly, pulses like field pea and cowpea which are easily available sources of phenolics have not been explored for their anti-diabetic potential. Moreover, many of the health-beneficial effects of pulses arise in part through the antioxidants present in them that protect against oxidative stress (Velderrain *et al*. 2014, Bielli *et al*. 2015). Phenolic compounds, including phenolic acids, flavonoids, and tannins, are found in high quantities in pulses and have excellent antioxidant properties (Amarowicz and Pegg 2008).

As a result of their diverse health benefits, the research on phenolic compounds from diverse sources has witnessed ever-increasing attention. The phenolic content and composition differ among different pulse crops and cultivars. However, a comprehensive investigation of phenolic diversity, antioxidant properties, and the nutraceutical potential of field pea and cowpea cultivars has not been reported in scientific literature. Hence, to bridge this existing research gap the present study aims to investigate the variation in phenolic content, phenolic profile, antioxidant potential, and their α-amylase and α-glucosidase inhibition potential of diverse field pea and cowpea cultivars.

MATERIALS AND METHODS

Plant materials

Fifteen field pea cultivars (IPF 99-25, IPF 5-19, IPFD 10-12, IPFD 1-10, IPFD 12-2, IPFD 2014-2, Arkel, Azad P-3, IPF 16-13, P-1586, P-489, IPF 12-20, EC-328758, EC-341792 and B-22) having diverse seed coat color and twelve cowpea cultivars (TPTC 29, TCS 160, GC 901, TC 901, KBC 7, KBC 9, PL 2, PL 3, DC 15, DC 16, GC 3, and RC 101) of diverse seed coat colour used for this study were procured from All India Coordinated Research Project on MuLLaRP, ICAR-IIPR, Kanpur (Table 1). The seeds were ground to a fine powder and stored in airtight packets at 4°C until use.

Chemicals and reagents

Propyl gallate, ethanol, Folin-Ciocalteau reagent, tripyridyl-S-triazine (TPTZ), ferric chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Neocuproine, sodium acetate, sodium carbonate, ferrous sulphate heptahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX), methanol, hydrochloric acid, *Saccharomyces cerevisiae* α-glucosidase, p-nitrophenyl-α-D-glucopyranoside, di-nitro salicylic acid, porcine pancreatic α-amylase, maltose and HPLC grade gallic acid, proto-catechuic acid, p-coumaric acid, ferulic acid, caffeic acid, sinapic acid, ellagic acid, catechin hydrate, quercetin and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade methanol, trifluoroacetic acid and water were purchased from Merck Millipore.

Total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC)

Total phenols were extracted from 1 g defatted flour of each field pea and cowpea cultivar using 10 mL of 70% ethanol and shaking the resultant slurry for 3 hours at 300 rpm on a shaker (Scientific Industries Inc., USA) at room temperature. It was then centrifuged at 3000 g for 15 minutes (Sigma 2-16KL Bench-top Refrigerated Centrifuge) and the supernatant of each cultivar was collected. The residue was re-extracted twice using 10 ml of 70% ethanol. The supernatants of each were collected and pooled in amber colour bottles and stored in the dark at 4°C until use. The total phenolic content was estimated using the Folin-Ciocalteau method with slight modifications as suggested by Xu and Chang (2007). Briefly, 50 µL ethanolic extract of each genotype was added to a test tube, followed by the addition of 3 mL double distilled water, 250 µL Folin-Ciocalteau reagent, and 750 µL of 7% sodium carbonate (Na_2CO_3) solution. The contents were mixed well and incubated for 2 hours at room temperature before measuring absorbance at 765 nm against distilled water as blank. A standard curve was simultaneously prepared using different concentrations of gallic acid. The calibration curve was linear in the range of 20 to100 µg/ml. Results were expressed in terms of mg gallic acid equivalent

(GAE)/gram seed.

To extract total flavonoids from field pea and cowpea cultivars, 1 g defatted flour of each cultivar was extracted with 10 ml of 70% methanol containing 0.1% HCl (v/v). The extraction was carried out at 300 rpm on a rotary shaker (Scientific Industries Inc., USA) for 3 hours in the dark and at room temperature. It was then centrifuged at 6000 rpm for 15 minutes and the supernatant of each cultivar was collected in amber bottles. The pellets were re-extracted with 10 ml of 70% methanol containing 0.1% HCl (v/v) and supernatants were pooled and stored at 4°C until used. The total flavonoids were quantified using the aluminum chloride assay as described by Kim *et al.* (2003). Briefly, 500 µL extract was taken in a test tube and the volume was made to 5 ml with distilled water. Next, 0.3 ml of 5% $NaNO_2$ was added to it and after 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. Six minutes later, 2 ml of NaOH was added followed by dilution with 2.4 ml of distilled water. Absorbance was measured at 510 nm against the sample blank. A standard curve of catechin ranging from 5 to 200 µg was prepared simultaneously. Results were expressed in units of mg catechin equivalent (CE)/g seed.

To extract condensed tannins from field pea and cowpea cultivars, 100 mg of defatted flour of each cultivar was extracted with 10 ml of double distilled water and boiled for 30 minutes in a water bath. The supernatants were collected after centrifugation at 2000 rpm for 20 minutes. The condensed tannins were quantified using the method given by Burns (1963). Briefly, 100 µl of each extract was transferred to a test tube and the volume was made to 1000 µl with double distilled water. Next, 1 ml of Folin Denis reagent was added followed by 2 ml of sodium carbonate (Na_2CO_3) solution. After 30 minutes of incubation at room temperature, the absorbance was noted at 700 nm. A standard curve of tannic acid ranging from 0 to 100 µg was prepared simultaneously. Results were expressed in terms of mg Tannic Acid Equivalent (TAE)/g seed.

Total antioxidant activity by FRAP, DPPH and CUPRAC assay

To evaluate the total antioxidant activity of field pea and cowpea cultivars, 1 g defatted flour from each cultivar was extracted for 3 hours with 10 ml of 70% methanol containing 0.1% HCl (v/v). The extraction was carried out at 300 rpm on a rotary shaker at room temperature. The resultant slurry was then centrifuged at 6000 rpm for 15 minutes. The supernatants were collected in amber bottles and the pellet was re-extracted with 10 ml of 70% methanol containing 0.1% HCl (v/v). The supernatants were pooled and stored at 4°C until used.

Ferric-reducing antioxidant power (FRAP) assay

The antioxidant capacity of each field pea and cowpea cultivar was determined using the FRAP method as outlined by Thaipong *et al.* (2006). FRAP reagent was freshly prepared by mixing 50 ml of 300 mM, pH 3.6 acetate buffer, 5 ml of 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution prepared in 40 mM HCl, and 5 ml of 20 mM $FeCl_3.6H_2O$ solution. In a test tube, 100 µL of each methanolic sample extract was taken and the volume was made to 1 ml with 300 mM, pH 3.6 acetate buffer. Next, 2.5 ml of FRAP reagent was added and the contents were mixed well after which the test tubes were kept in the dark for 10 minutes. Absorbance was recorded at 593 nm against the sample blank. A standard curve of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) with concentrations ranging from 50 µM-1000 µM was prepared simultaneously. Results were expressed in units of µmol Fe (II) equivalent/g DW.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was carried out according to the method given by Brand-Williams *et al.* (1995). Briefly, 50 µl of methanolic sample extract was added to 3.9 ml of 0.06 mM DPPH solution prepared in 95% methanol. The mixture was vortexed and incubated in dark for 30 minutes. Absorbance was recorded at 517 nm against 95% methanol as blank. The DPPH inhibition percentage was calculated using the formula:

DPPH radical scavenging capacity % = [Ac-(As-Asb) ÷ Ac] × 100

where Ac is the absorbance of the control reaction (containing only DPPH), As is the absorbance of the sample (containing sample extract and DPPH) and Asb is the absorbance of the sample blank (containing only sample extract). Results were expressed in units of µmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX) equivalent/g DW.

Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC assay was done as described by Apak *et al.* (2007). Briefly, 50 µl of methanolic sample extract was added to a test tube and it was followed by the addition of 1 ml each of 0.01 M Cu (II)

chloride solution, 7.5×10^{-3} M Neocuproine solution prepared in 95% ethanol and ammonium acetate buffer (pH 7.0). The reaction mixture was incubated at room temperature for 1 hour and absorbance was recorded at 450 nm against a reagent blank. Results were expressed in units of µmol TROLOX equivalent/g DW.

High-performance liquid chromatography-based phenolic profiling

Extraction of free phenolics from field pea and cowpea samples was done as described by Ross, Beta, and Arntfield (2009) with slight modifications. Briefly, 1.5 g flour from each cultivar was extracted with 7.5 ml of 70% methanol containing 0.1% HCl in an ultrasonic bath for 1 hour at 40 °C. It was then kept for shaking at 250 rpm for 2 hours at 37 °C. The supernatant was collected after centrifugation at 8000 rpm for 15 minutes. Extraction was repeated and the supernatants were pooled and evaporated in a water bath at 45 °C. The dried extracts were dissolved in 1.5 ml methanol and centrifuged at 10000 rpm for 10 minutes, and the supernatant was filtered through a 0.2 µ nylon filter. The filtered extract stored in amber vials was used for HPLC analysis of free phenolics.

For the extraction of bound phenolics, 20 ml of 2N NaOH containing 10 mM EDTA and 0.1 % ascorbic acid was added to the pellet obtained after the extraction of free phenolics. The resultant slurry was left overnight at room temperature. Centrifugation was done at 10000 rpm for 10 minutes and the pH of each supernatant was adjusted to 2.0 with 6 N HCl. Next, 10 ml ethyl acetate and diethyl ether mixture (50:50 v/v) was added and vortexed vigorously. Supernatants were collected after centrifugation at 10000 rpm for 10 minutes and evaporated in a water bath at 40 °C. The dried extracts were dissolved in 1.5 ml methanol and centrifuged at 10000 rpm for 10 minutes. The supernatants were filtered with a 0.2 µ nylon filter and stored in amber vials for analysis of bound phenolics.

Chromatographic analysis was done on an Agilent model (1260 infinity) equipped with a diode array detector. The absorbance was measured at 238 and 254 nm. The mobile phase consisted of 0.1% trifluoracetic acid in acetonitrile (solvent A), 0.1% trifluoroacetic acid in HPLC grade water (solvent B), and 100% methanol (solvent C). The flow rate of the mobile phase was 1ml/minute, and the column temperature was maintained at 37°C. The gradient used was 100% solvent B, 0% solvent A at 0 minutes, 90% solvent B and 10% solvent A at 0-7 minutes, 90% solvent B and 10% solvent A at 7-10 minutes, 60% solvent B and 40% solvent A at 10-30 minutes, 60% solvent B and 40% solvent A at 30-32 minute, and 100% solvent B and 0% solvent A at 32-35 minute. Solvent C was used for column washings.

The linearity, repeatability, limits of detection (LOD), and limit of quantification (LOQ) were determined for the HPLC-DAD method used (Table 1). The calibration curve was linear for the ten phenolic standards and the correlation coefficients exceeded 0.99 for each standard. The LOD values ranged from 2.04 to 4.77 µg/ml, while the LOQ values varied from 6.18 to 14.45 µg/ml indicating good resolution and sensitivity.

α-amylase inhibitory activity

To study the inhibition of α-amylase activity by field pea and cowpea phenolic extracts the protocol described by Sigma Aldrich was followed with slight modifications. Briefly, the reaction mixture containing 25 µl of sample phenolic extract and 200 µl of porcine pancreatic α-amylase (1Unit/ml in cold deionized water) was incubated for 20 minutes at 37°C. This was followed by the addition of 100 µl of soluble starch solution (1% w/v in 20 mM sodium phosphate buffer containing 6.7 mM sodium chloride, pH 6.9 and the reaction was incubated for 10 minutes. The reaction was terminated by adding 100 µl of DNS color reagent (mix 8 ml of warm 5.3 M potassium sodium tartrate tetrahydrate in 2 M NaOH, 12 ml of warm ultrapure water, and 20 ml of warm 96 mM 3,5-Dinitrosalicylic acid solution) and kept in boiling water bath for 10-15 minutes. It was then cooled on ice and diluted by adding 560 µl of double distilled water. A maltose standard was also prepared (ranging from 40 µg to 200 µg) from 0.2% w/v stock solution of maltose. Acarbose was used as a positive control. Results were expressed in terms of α-amylase inhibition unit/mg seed. One α-amylase inhibition unit was defined as the amount of enzyme that liberates 1µg of maltose under assay conditions.

α-glucosidase inhibitory activity

To study the inhibition of α-glucosidase activity by field pea and cowpea extracts the method described by Johnson *et al*. (2011) was followed with slight modifications. Briefly, the reaction mixture containing 250 µl of sample phenolic extract, 400 µl sodium phosphate buffer (100 mM, pH 6.8), and 25 µl *Saccharomyces cerevisiae* α-glucosidase (1

unit/ml in ice-cold sodium phosphate buffer) was incubated at 37°C for 10 minutes. Next, 30 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in sodium phosphate buffer (100 mM, pH 6.8) was added as a substrate and the reaction was incubated at 37°C for 15 minutes. The reaction was terminated by adding 250 µl of 0.1 M sodium carbonate. Along with the sample, control (no sample extract), sample blank (no enzyme), and blank reaction (no substrate) mixtures were also prepared. Acarbose, a common inhibitor of α-glucosidase was used as a positive control. Results were expressed in terms of α-glucosidase inhibition unit/mg seed. One unit of α-Glucosidase activity was defined as the amount of enzyme that liberates 1 µM of p-nitrophenol from p-nitrophenyl α-D-glucopyranoside.

Statistical analysis

Statistical analysis was carried out with JMP. All samples were analyzed in three replicates. The results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) with posthoc Tukey test was conducted to determine significant differences between different cultivars. Means were considered statistically different at p-value <0.05. Correlations among various parameters were examined using Pearson's correlation coefficient. The data on antioxidant activity and phenolic compounds were subjected to principal component analysis (PCA). Hierarchical cluster analysis was performed by taking the means of TPC, TFC, CTC, FRAP, DPPH, and CUPRAC values.

RESULTS AND DISCUSSION

Total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin content (CTC)

Phenolic acids, flavonoids, and tannins are the major phenolic compounds present in pulses (Campos-Vega *et al.* 2010). The total phenolic content (TPC), total flavonoid content (TFC), and condensed tannin content (CTC) of field pea and cowpea cultivars investigated here are enlisted in Table 1 and expressed as mg gallic acid equivalent (GAE)/g seed, mg catechin equivalent (CE)/g seed and mg tannic acid equivalent (TAE)/g

Table 1. Total phenolic content, total flavonoid content, and condensed tannin content of field pea and cowpea cultivars

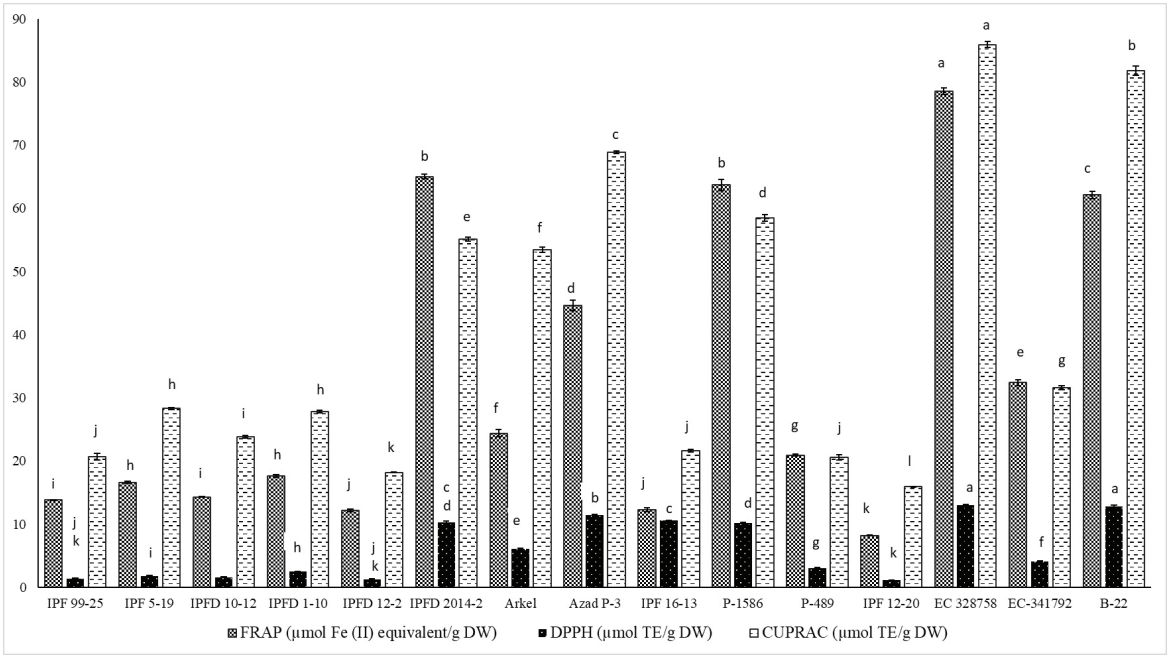
Field pea cultivars	**TPC[b]**	**TFC[c]**	**CTC[d]**
IPF 99-25	1.22 ± 0.00^{k}	0.17 ± 0.00^{h}	0.80 ± 0.03^{hi}
IPF 5-19	1.31 ± 0.01^{i}	0.14 ± 0.00^{j}	0.70 ± 0.02^{i}
IPFD 10-12	1.13 ± 0.00^{l}	0.20 ± 0.01^{e}	0.70 ± 0.01^{i}
IPFD 1-10	1.41 ± 0.02^{h}	0.17 ± 0.00^{h}	0.70 ± 0.01^{i}
IPFD 12-2	1.27 ± 0.01^{j}	0.14 ± 0.00^{k}	1.00 ± 0.03^{fg}
IPFD 2014-2	2.15 ± 0.04^{e}	0.21 ± 0.00^{c}	1.80 ± 0.03^{c}
Arkel	1.74 ± 0.02^{f}	0.19 ± 0.01^{f}	0.90 ± 0.02^{gh}
Azad P-3	2.71 ± 0.05^{b}	0.18 ± 0.00^{g}	1.30 ± 0.02^{e}
IPF 16-13	1.13 ± 0.00^{l}	0.16 ± 0.00^{i}	0.70 ± 0.02^{i}
P-1586	2.23 ± 0.03^{d}	0.27 ± 0.01^{b}	1.60 ± 0.03^{d}
P-489	0.92 ± 0.01^{m}	0.16 ± 0.00^{i}	0.80 ± 0.02^{hi}
IPF 12-20	0.58 ± 0.01^{n}	0.13 ± 0.00^{l}	0.70 ± 0.03^{i}
EC-328758	2.74 ± 0.03^{a}	0. 35± 0.01^{a}	2.40 ± 0.10^{a}
EC-341792	1.68 ± 0.03^{g}	0.21 ± 0.01^{d}	1.10 ± 0.10^{f}
B-22	2.58± 0.02^{c}	0.20± 0.00^{d}	2.00 ± 0.10^{b}
Cowpea cultivars	**TPC[b]**	**TFC[c]**	**CTC[d]**
TPTC 29	4.41 ± 0.10^{g}	0.85 ± 0.02^{e}	2.4 ± 0.22^{c}
TCS 160	2.12 ± 0.01^{j}	0.32 ± 0.00^{k}	0.7 ± 0.01^{e}
GC 901	7.14 ± 0.11^{a}	1.67 ± 0.01^{a}	3.2 ± 0.05^{a}
TC 901	2.69 ± 0.01^{i}	0.89 ± 0.01^{d}	0.8 ± 0.02^{de}
KBC 7	4.95 ± 0.10^{c}	1.04 ± 0.02^{c}	2.4 ± 0.05^{c}
KBC9	4.56 ± 0.10^{f}	0.79 ± 0.00^{f}	2.8 ± 0.01^{b}
PL 2	4.64 ± 0.07^{d}	0.60 ± 0.01^{h}	1.0 ± 0.01^{d}
PL 3	6.06 ± 0.11^{b}	1.35 ± 0.04^{b}	3.0 ± 0.12^{a}
DC 15	4.62 ± 0.08^{e}	0.72 ± 0.01^{g}	1.0 ± 0.02^{d}
DC 16	3.88 ± 0.01^{h}	0.41 ± 0.00^{j}	0.7 ± 0.01^{e}
GC 3	1.91 ± 0.00^{k}	0.58 ± 0.02^{i}	0.3 ± 0.00^{f}
RC 101	1.80 ± 0.01^{l}	0.26 ± 0.01^{l}	0.7 ± 0.02^{e}

[a]Values are mean ± SD, n=3. Values followed by the different letter in the same column are significantly different (P < 0.05) by the Tukey's test.
[b]Total Phenolic Content (TPC). Values are expressed as mg Gallic Acid Equivalent/g seed.
[c]Total Flavonoid Content (TFC). Values are expressed as mg Catechin Equivalent/g seed.
[d]Condensed Tannin Content (CTC). Values are expressed as mg Tannic Acid Equivalent/g seed.

Table 1. Retention times (Rt), absorbance wavelengths (λ), regression equations, linearity (R^2), limits of detection (LODs) and limits of quantification (LOQs) of the standard phenolic analytes. Results are mean of three independent experiments.

	Analytes	**Rt (min)**	**λ (nm)**	**Regression equations**	**R^2**	**LODs (µg/ml)**	**LOQs (µg/ml)**
1	Gallic acid	3.9	254	y = 27.234x - 75.078	0.993	2.87	8.70
2	Protocatechuic acid	5.2	254	y = 54.261x - 9.5509	0.995	3.16	9.58
3	Catechin hydrate	8.4	238	y = 30.945x - 2.9964	0.996	4.35	13.18
4	Caffeic acid	9.1	238	y = 48.999x + 11.215	0.999	4.15	12.58
5	p-Coumaric acid	11.8	238	y = 34.226x - 18.251	0.999	4.56	13.82
6	Ferulic acid	14.2	238	y = 58.617x - 52.89	0.999	4.48	13.58
7	Sinapic acid	15.2	238	y = 76.69x - 83.25	0.998	4.77	14.45
8	Ellagic acid	17.1	272	y = 183.37x - 95.853	0.999	2.26	6.85
9	Quercetin	22.3	272	y = 70.678x – 52.894	0.999	2.67	8.11
10	Kaempferol	25.0	272	y = 64.52x – 73.378	0.999	2.04	6.18

(a)



(b)

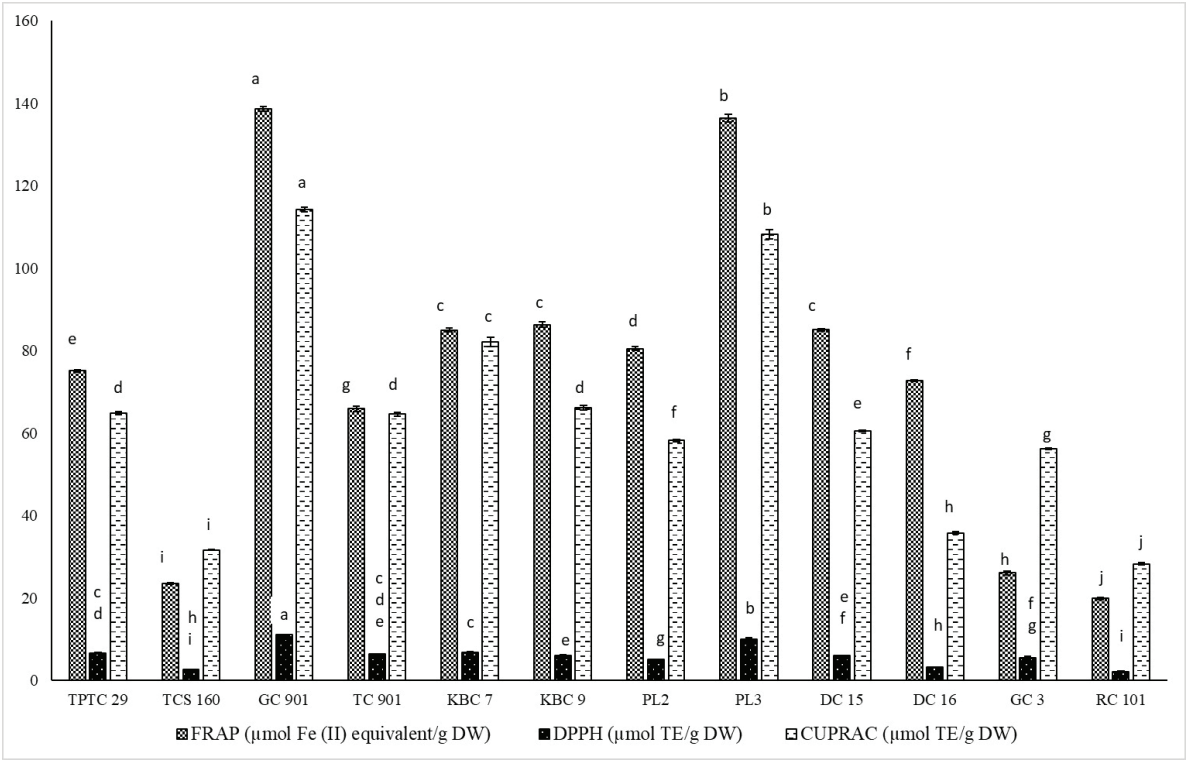
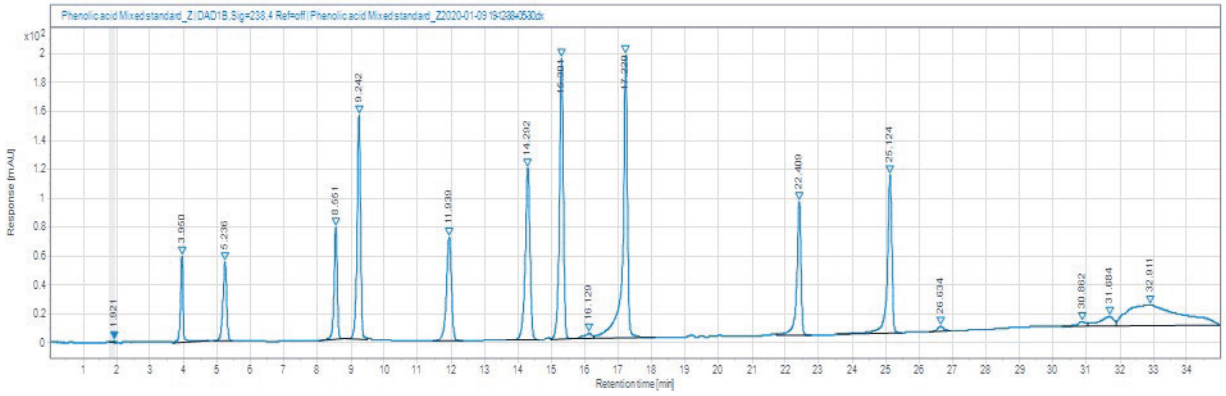


Fig. 1. Antioxidant activities of fifteen different cultivars of field pea (a), and twelve different cultivars of cowpea (b). FRAP, DPPH, and CUPRAC are the three methods used to determine antioxidant activities. FRAP values are expressed as µmol Fe (II) equivalent/g DW; DPPH and CUPRAC values are expressed as µmol Trolox equivalent (TE)/g DW. All values are means ± standard deviation (n=3). Columns in panels A and B with different letters indicate significant differences ($p < 0.05$) by Tukey's test for the same antioxidant assay.

(a)



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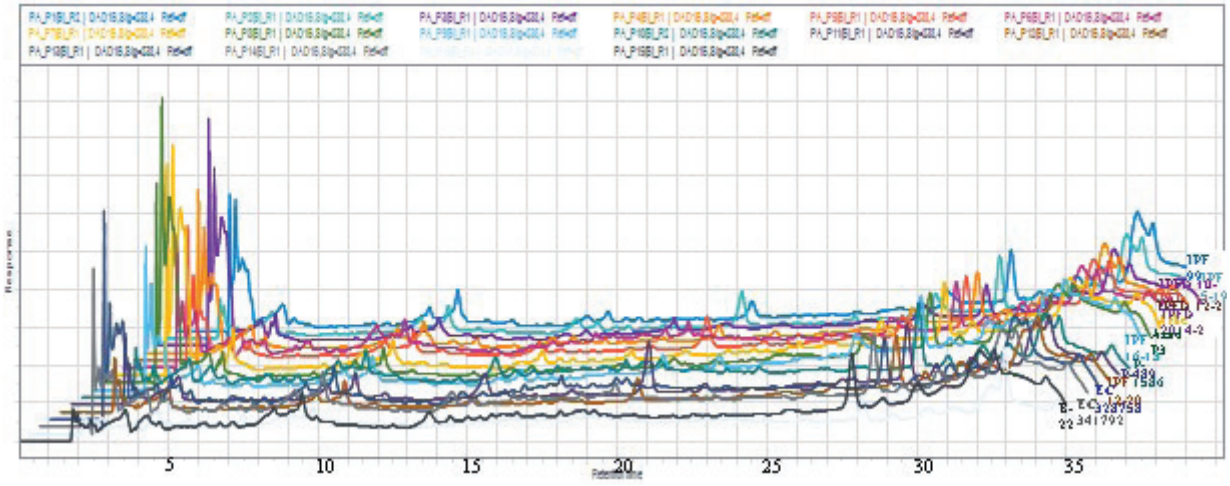

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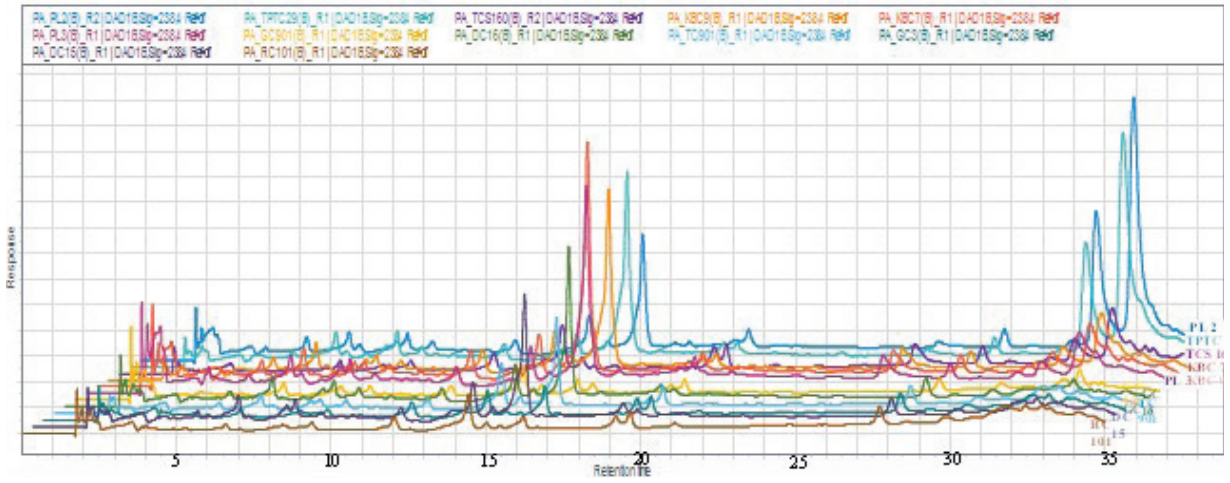
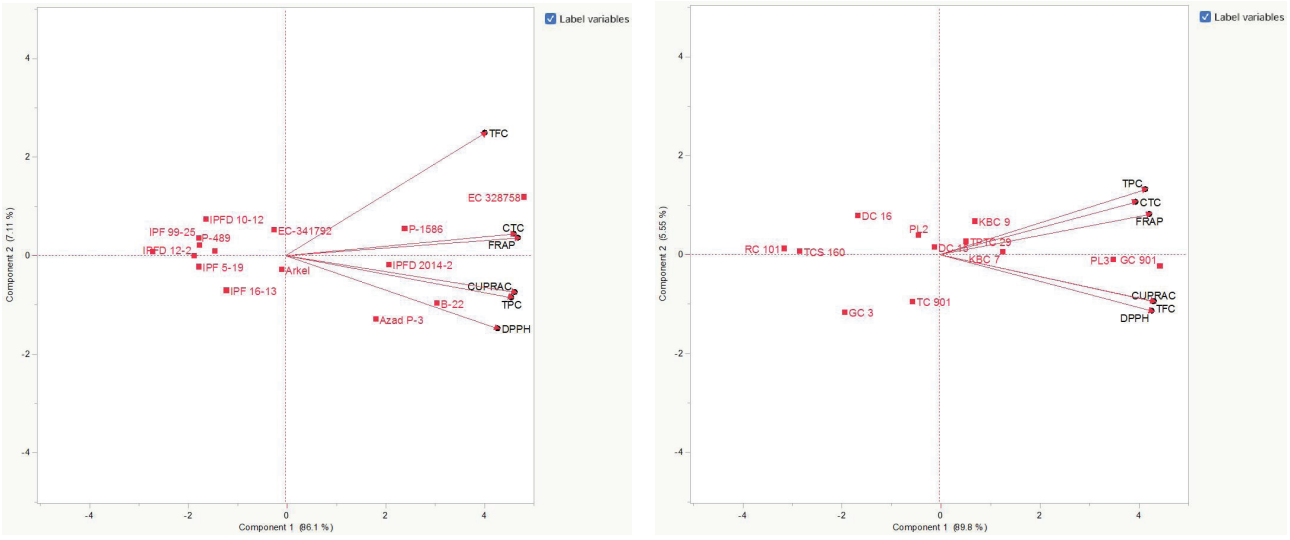


Fig. 2. Chromatogram depicting retention time of ten phenolic standards (a), bound phenolics in fifteen field pea cultivars (b), and bound phenolics in twelve cowpea cultivars (c).

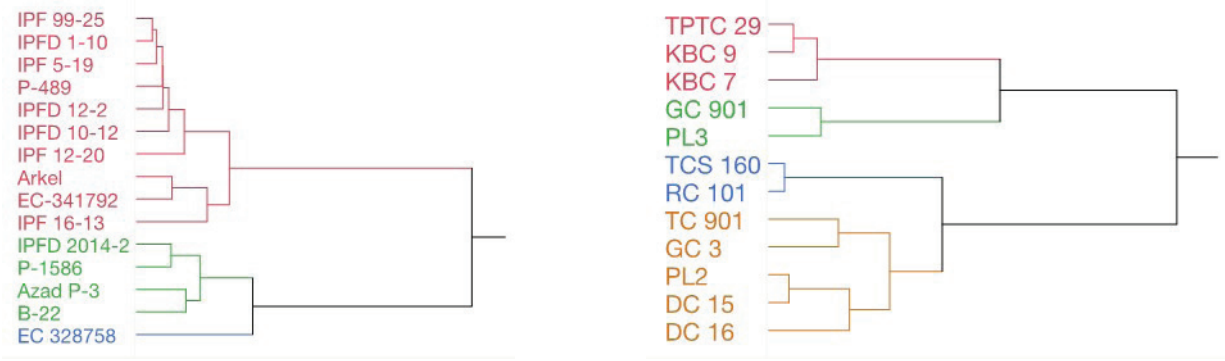
seed respectively. The TPC, TFC, and CTC values varied significantly ($p < 0.05$) among field pea and cowpea cultivars. In field pea cultivars these values varied from 0.58 to 2.74 mg GAE/g seed, 0.13 to 0.35 mg CAE/g seed, and 0.47 to 2.4 mg TAE/g seed respectively. The reddish-brown cultivar EC 328758 showed the highest TPC, TFC as well as CTC whereas the green-seeded cultivar IPF 12-20 showed the least TPC, TFC, and CTC. As all the cultivars were grown under the same ecological conditions and harvested at the same stage, the variation in TPC, TFC, and CTC values among cultivars could be attributed to genetic differences among them. It was observed that the field pea cultivars with brown and black seed coat colors have higher TPC, TFC, and CTC than the cultivars with yellow and green seed coat colors. Our finding that green peas have lesser total phenolic content than yellow peas is also supported by Xu *et al*. (2007) who reported similar results. The analysis of total phenolic content in ten differently colored European field pea cultivars by Stanisavljević *et al*. (2016) also concluded that the cultivars with dark seed coats have higher phenolic content than cultivars with light seed coat color.

The TPC, TFC, and CTC values of cowpea cultivars varied from 1.8 to 7.14 mg GAE/g seed, 0.26 to 1.67 mg CAE/g seed, and 0.3 to 3.2 mg CAE/g seed respectively. The golden-brown cowpea cultivar GC 901 showed the highest TPC, TFC, and CTC whereas the white-seeded cultivar RC 101 showed the least TPC and TFC content. The lowest CTC value was noted in the yellowish-brown cultivar GC 3. The analysis of TPC and



(a) (b)

Fig. 3. PCA biplot of phenolic compounds and antioxidant activity of fifteen field pea cultivars (a), and twelve cowpea cultivars (b).



(a) (b)

Fig. 4. Dendrogram of hierarchical cluster analysis of fifteen field pea cultivars (a), and twelve cowpea cultivars (b).

Table 2. Composition of phenolic acids and flavonoids in the seed of fifteen field pea cultivars

Compound	IPF 99-25	IPF 5-19	IPFD 10-12	IPFD 1-10	IPFD 12-2	IPFD 2014-2	Arkel	Azad P-3	IPF 16-13	P-1586
Gallic acid	7.24 ±0.09^{f}	7.80 ±0.08^{e}	11.67±0.32^{b}	3.77±0.11^{h}	7.57±0.20^{ef}	6.43±0.05^{g}	10.71±0.14^{c}	13.8±0.23^{a}	9.87±0.24^{d}	9.80±0.05^{d}
Proto-catechuic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.19±0.01^{b}
p-Coumaric acid	ND	ND	4.85±0.13^{b}	5.66±0.11^{a}	ND	ND	ND	ND	ND	ND
Ferulic acid	ND	ND	ND	0.48±0.04^{a}	ND	ND	0.58±0.06^{a}	0.45±0.05^{a}	0.51±0.04^{a}	ND
Caffeic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sinapic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ellagic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Catechin hydrate	3.16 ±0.05^{cd}	3.1 ±0.02^{cd}	0.49 ±0.03^{h}	0.58 ±0.00^{h}	1.01 ±0.02^{g}	7.41 ±0.14^{a}	3.32 ±0.05^{c}	3.32 ±0.03^{c}	2.36 ±0.01^{f}	3.39±0.07^{b}
Quercetin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kaempferol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Compound	**P-489**	**IPF 12-20**	**EC 328758**	**EC 341792**	**B-22**					
Gallic acid	7.24 ±0.09^{f}	7.96±0.12^{e}	14.19±0.25^{a}	13.80±0.22^{a}	12.07±0.26^{b}					
Proto-catechuic acid	ND	ND	0.26 ±0.01^{a}	0.22 ±0.02^{b}	ND					
p-Coumaric acid	ND	ND	ND	ND	ND					
Ferulic acid	ND	ND	ND	ND	ND					
Caffeic acid	ND	ND	ND	ND	ND					
Sinapic acid	ND	ND	ND	ND	ND					
Ellagic acid	ND	ND	ND	ND	ND					
Catechin hydrate	3.16 ±0.05^{cd}	2.69 ±0.04^{e}	2.30 ±0.01^{f}	2.47 ±0.03^{ef}	3.01 ±0.05^{d}					
Quercetin	ND	ND	ND	ND	ND					
Kaempferol	ND	ND	ND	ND	ND					

aValues are expressed as mg/100 g seed.
Values are mean ± SD, n=3. Values followed by the different letter in the same row are significantly different ($P < 0.05$) by the Tukey's test.

TFC in 31 genotypes of cowpeas by Sombie *et al*. (2018) also reported a TPC and TFC range similar to our findings. However, a recent study of cowpea varieties grown in Ethiopia by Teka *et al.* (2020) reported much higher TPC (12.1 to 16.1 mg GAE/g flour on a dry matter basis) and TFC (2.5 to 11.1 mg GAE/g flour on a dry matter basis) values which could be due to genetic and environmental differences.

Overall, the results show that in comparison to field pea, the cowpea cultivars have higher TPC, TFC, and CTC values. The average sum of TPC, TFC, and CTC of cowpea cultivars studied here was 2.46, 4.15, and 0.13 times the average sum of TPC, TFC, and CTC of field pea cultivars respectively.

Total antioxidant activity by FRAP, DPPH and CUPRAC assay

The estimation of the antioxidant potential of pulses is important from a nutraceutical perspective. The results of the *in vitro* antioxidant activity of field pea and cowpea cultivars estimated through FRAP, DPPH and CUPRAC assays are presented in Fig. 1. The FRAP values of field pea cultivars ranged from 8.2 ± 0.06 µmol Fe (II) equivalent/g DW in green seeded cultivar IPF 12-20 to 78.5 ± 0.51 µmol Fe (II) equivalent/g DW in the reddish-brown cultivar EC 328758 (Fig. 1a). Statistically, the FRAP values differed significantly among the cultivars ($p < 0.05$). As compared to field peas, the cowpea cultivars showed much higher FRAP value which varied from 19.9 ± 0.25 µmol Fe (II) equivalent/g DW in the white-seeded cultivar RC 101 to 138.6 ± 0.62 µmol Fe (II) equivalent/g DW in the golden-brown cultivar GC 901 (Fig. 1b). The DPPH radical scavenging activity of field pea cultivars ranged from 8.2 µmol Trolox equivalent/g DW in the green seeded cultivar IPF 12-20 to 13.0 µmol Trolox equivalent/g DW in the reddish-brown cultivar EC 328758 (Fig. 1a). In cowpeas, the DPPH radical scavenging activity ranged from 2.2 µmol Trolox equivalent/g DW in the white-seeded cultivar RC 101 to 11.1 µmol Trolox equivalent/g DW in the golden-brown cultivar GC 901 (Fig. 1b). The CUPRAC activity of field pea cultivars ranged from 15.9 µmol Trolox equivalent/g DW in green-seeded IPF 12-20 to 85.9 µmol Trolox equivalent/g DW in the reddish-brown cultivar EC 328758 (Fig. 1a). Compared to field pea the cowpea cultivars exhibited higher CUPRAC activity which varied from 28.3 µmol Trolox equivalent/g DW in white-seeded RC 101 to 114.2 µmol Trolox equivalent/g DW in the golden-brown cultivar GC 901 (Fig. 1b).

Our results of the antioxidant activity assay show that field pea cultivars with reddish-brown, dark brown, and black seed coat colors have stronger antioxidant activity than cultivars with yellow and green seed coat colors. Likewise, the golden-brown and reddish-brown cultivars of cowpea have stronger antioxidant activity than the yellowish-white and white-seeded cultivars. Also, the FRAP, DPPH, and CUPRAC values of field pea and cowpea cultivars showed a strong positive correlation with their TPC, TFC, and CTC content (Table 5 & 6) which suggests the contribution of phenolics to the antioxidant activity of these pulses. The positive correlation of phenolics with total antioxidant capacity has been well confirmed in other pulse crops as well (Amarowicz *et al*. 2004, Xu and Chang 2007, Yeo and Shahidi 2015).

Phenolic profile of field pea and cowpea cultivars

In nature, phenolic acids are mostly present in insoluble bound form and flavonoids in the form of glycosides. The identification of free and bound phenolics of field pea and cowpea cultivars was done by comparing the retention time and UV-visible spectra to those of suitable HPLC grade standards (Fig. 2a). Chromatogram depicting phenolic profile of field pea and cowpea cultivars are shown in Fig. 2b & 2c. Quantitative results of phenolic acids and flavonoids identified in field pea and cowpea cultivars are presented in Tables 2 & 3 respectively and are expressed in terms of mg/100 g seed. Overall, two hydroxybenzoic acid derivatives (gallic acid and protocatechuic acid), two hydroxycinnamic acid derivatives (p-coumaric acid and ferulic acid), and a flavan 3-ol (catechin hydrate) were identified in field pea cultivars. The gallic acid in free form was present in all the fifteen field pea cultivars and its highest concentration was detected in the reddish-brown cultivar EC 328758 (14.19 mg/100 g seed). The free, as well as bound form of protocatechuic acid, was detected in only three cultivars namely EC 328758 (0.26 ± 0.01 mg/100 g seed), EC 341792 (0.22 ± 0.02 mg/100 g seed) and the black seeded cultivar P-1586 (0.19 ± 0.01 mg/100 g seed). The p-coumaric acid was also detected in bound form in only two cultivars, the green seeded IPFD 10-12 (4.85 ± 0.13 mg/100 g seed) and the dark yellow IPFD 1-10 (5.66 ± 0.11 mg/100 g seed). Ferulic acid in free, as well as bound form, was found in IPFD 1-10 (0.48 ± 0.04 mg/100 g seed), green seeded IPF 16-13 (0.51 ± 0.04 mg/100 g seed), and the wrinkled type cultivars Arkel (0.58 ± 0.06 mg/100 g seed) and Azad P-3 (0.45 ± 0.05 mg/100

Table 3. Composition of phenolic acids and flavonoids in the seed of twelve cowpea cultivars

Compound	TPTC 29	TCS 160	GC 901	TC 901	KBC 7	KBC 9	PL 2	PL 3	DC 15	DC 16
Gallic acid	6.68 ±0.03^{g}	11.69 ±0.15^{c}	7.81±0.12^{f}	6.34 ±0.12^{g}	8.00 ±0.04^{ef}	8.00 ±0.06^{ef}	9.54 ±0.12^{d}	8.36 ±0.05^{e}	8.03 ±0.1^{ef}	12.37 ±0.21^{b}
Proto-catechuic acid	1.89 ±0.01^{e}	1.68 ±0.01^{f}	1.12 ±0.02^{gh}	2.43 ±0.03^{d}	4.82 ±0.12^{a}	4.31 ±0.07^{b}	4.97 ±0.1^{a}	1.79 ±0.12^{ef}	2.71 ±0.04^{c}	2.78 ±0.05^{c}
p-Coumaric acid	3.17 ±0.08^{c}	4.20 ±0.1^{b}	2.61±0.14^{ef}	2.02 ±0.03^{g}	5.06 ±0.34^{a}	4.56 ±0.25^{b}	3.12 ±0.05^{c}	4.53 ±0.1^{b}	2.39 ±0.07^{fg}	2.64±0.04^{def}
Ferulic acid	2.66 ±0.04^{fg}	4.90 ±0.04^{a}	3.35 ±0.13^{def}	4.78±0.15^{ab}	3.84 ±0.13^{cd}	3.77 ±0.23^{cde}	3.96±0.12^{cd}	4.08 ±0.23^{c}	3.14 ±0.45^{ef}	3.03 ±0.50^{fg}
Caffeic acid	0.13 ±0.01^{c}	ND	ND	0.92 ±0.02^{b}	ND	ND	ND	ND	0.77 ±0.05^{bc}	0.72 ±0.01^{bc}
Sinapic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ellagic acid	ND	0.20 ±0.02^{e}	ND	0.68 0.04^{b}	0.30±0.01^{d}	0.29 ±0.01^{d}	1.06 ±0.02^{a}	ND	0.25 ±0.01^{de}	0.22 ±0.02^{e}
Catechin hydrate	2.71 ±0.05^{e}	2.22 ±0.04^{f}	7.49 ±0.23^{a}	3.13 ±0.15^{d}	4.81 ±0.07^{bc}	4.44 ±0.12^{c}	4.96 ±0.14^{b}	7.41 ±0.23^{a}	3.31 ±0.10^{d}	3.21 ±0.08^{d}
Quercetin	0.32 ±0.04^{e}	0.36 ±0.06^{cde}	0.29±0.04^{e}	0.43 ±0.05^{bcd}	0.45 ±0.05^{bc}	0.44 ±0.03^{bcd}	1.09 ±0.02^{a}	0.34 ±0.01^{de}	0.32 ±0.02^{e}	0.39 ±0.03^{cde}
Kaempferol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Compound	**GC 3**	**RC 101**								
Gallic acid	9.47 ±0.15^{d}	12.80 ±0.22^{a}								
Proto-catechuic acid	1.17 ±0.01^{g}	0.96 ±0.06^{h}								
p-Coumaric acid	3.05 ±0.09^{cd}	2.94 ±0.06^{cde}								
Ferulic acid	2.45 ±0.01^{g}	4.21 ±0.05^{bc}								
Caffeic acid	ND	25.82 ±0.54^{a}								
Sinapic acid	ND	ND								
Ellagic acid	0.42 ±0.03^{c}	ND								
Catechin hydrate	7.49 ±0.23^{a}	0.48 ±0.02^{g}								
Quercetin	0.51 ±0.01^{b}	ND								
Kaempferol	ND	ND								

aValues are expressed as mg/100 g seed.
Values are mean ± SD, n=3. Values followed by the different letter in the same row are significantly different ($P < 0.05$) by the Tukey's test.

g seed). The flavonoid catechin was detected in all field pea cultivars in free as well as bound form and its maximum concentration was noted in the brown seeded cultivar IPFD 2014-2 (7.41 ± 0.14 mg/100 g seed). Other phenolic compounds like caffeic acid, sinapic acid, ellagic acid, quercetin, and kaempferol were not detected in field peas.

In comparison to field pea, the cowpea cultivars were found to have a more diverse phenolic profile. Overall, two hydroxybenzoic acid derivatives (gallic acid and protocatechuic acid), three hydroxycinnamic acid derivatives (p-coumaric acid, ferulic acid & caffeic acid), a polyphenol (ellagic acid), a flavan-3-ol (catechin hydrate) and a flavonol (quercetin) were detected in them. The free, as well as bound form of gallic acid, protocatechuic acid, p-coumaric acid, and ferulic acid, was detected in all the twelve cowpea cultivars. The highest concentration of gallic acid was noted in the white-seeded cultivar RC 101 (12.8 ± 0.22 mg/100g seed). The protocatechuic acid concentration was highest in the reddish-brown cultivar PL 2 (4.97 ± 0.1 mg/100g seed). The cultivar KBC 7 showed the highest p-coumaric acid concentration (5.06 ± 0.34 mg/100g seed). Likewise, the maximum concentration of ferulic acid was noted in the yellowish-white cultivar TCS 160 (4.9 ± 0.04 mg/100g seed). Caffeic acid was detected in only five cultivars of cowpea i.e. TPTC 29, TC 901, DC 15, DC 16, and RC 101. The polyphenol ellagic acid known for its bioactive properties was also detected in bound form in all cowpea cultivars except TPTC 29, GC 901, PL3, and RC 101. Quercetin was detected in bound form in all cowpea cultivars except the white-seeded RC-101. Its maximum concentration was found in the reddish-brown cultivar PL 2 (1.09 ± 0.02 mg/100g seed). Catechin was detected in free as well as bound form and its highest concentration was observed in the golden-brown cultivars GC 901 (7.49 ± 0.23 mg/100g seed) and PL 3 (7.41 ± 0.23 mg/100g seed). The least catechin content was noted in the white-seeded cultivar RC 101 (0.48 ± 0.02 mg/100g seed).

Overall, our study of the phenolic composition of field pea and cowpea cultivars showed that most of the phenolic compounds are present in a bound form and they get released upon hydrolysis with acid or alkali. Gallic acid and catechin hydrate were the predominant phenolic compounds in most of the field pea and cowpea cultivars (Tables 2 & 3). Similar to our results, Ojwang *et al*. (2013) also reported that cowpeas are abundant in the monomeric proanthocyanidin catechin. The

Table 4. Alpha amylase and alpha-glucosidase inhibitory activities of field pea and cowpea cultivars.

Name of genotype	Alpha amylase inhibitory activities (AAIU[b])	Alpha-glucosidase inhibitory activities (AGIU[c])
Field pea cultivars		
IPF 99-25	19.3 ± 0.18^{j}	0.42 ± 0.02^{d}
IPF 5-19	27.6 ± 0.69^{h}	0.28 ± 0.02^{fg}
IPFD 10-12	34.7 ± 0.12^{b}	0.32 ± 0.17^{ef}
IPFD 1-10	32.3 ± 0.65^{cde}	0.45 ± 0.01^{cd}
IPFD 12-2	30.7 ± 0.47^{g}	0.13 ± 0.01^{j}
IPFD 2014-2	25.3 ± 0.35^{i}	0.18 ± 0.01^{i}
Arkel	33.3 ± 0.43^{c}	0.58 ± 0.02^{a}
Azad P-3	36.3 ± 0.45^{a}	0.50 ± 0.03^{b}
IPF 16-13	31.0 ± 0.21^{fg}	0.33 ± 0.02^{e}
P-1586	31.3 ± 0.26^{efg}	0.24 ± 0.01^{gh}
P-489	32.0 ± 0.04^{def}	0.30 ± 0.01^{ef}
IPF 12-20	33.1 ± 0.59^{cd}	0.11 ± 0.00^{j}
EC-328758	31.6 ± 0.20^{efg}	0.55 ± 0.01^{a}
EC-341792	25.9 ± 0.32^{i}	0.23 ± 0.02^{hi}
B-22	33.5 ± 0.56^{bc}	0.48 ± 0.02^{bc}
Cowpea cultivars		
TPTC 29	30.55 ± 0.58^{c}	0.66 ± 0.01^{cde}
TCS 160	28.50 ± 0.36^{d}	0.73 ± 0.01^{bc}
GC 901	16.60 ± 0.20^{i}	1.97 ± 0.06^{a}
TC 901	22.85 ± 0.63^{f}	0.74 ± 0.04^{b}
KBC 7	31.20 ± 0.22^{c}	0.46 ± 0.02^{f}
KBC9	18.50 ± 0.22^{h}	0.68 ± 0.02^{bcd}
PL 2	26.55 ± 0.26^{e}	0.70 ± 0.03^{bcd}
PL 3	25.55± 0.51^{e}	0.74 ± 0.02^{b}
DC 15	30.65 ± 0.27^{c}	0.57 ± 0.01^{e}
DC 16	20.55 ± 0.40^{g}	0.60 ± 0.01^{e}
GC 3	36.80 ± 0.61^{a}	0.65 ± 0.03^{de}
RC 101	34.75 ± 0.71^{b}	0.40 ± 0.02^{f}

[a]Values are mean ± SD, n = 3. Values followed by the different letter in the same column are significantly different (P < 0.05) by the Tukey's test.

[b]Values are expressed as Alpha Amylase Inhibition Unit (AAIU)/mg seed. One unit of α-Amylase activity is defined here as the amount of α-Amylase that liberates 1µg of maltose under assay conditions.

[c]Values are expressed as Alpha Glucosidase Inhibition Unit (AGIU)/mg seed. One unit of α-Glucosidase activity is defined here as the amount of α-Glucosidase that liberates 1µM of p-nitrophenol from p-nitrophenyl α-D-glucopyranoside.

Table 5. Relationship between total phenolic content (TPC), total flavonoid content (TFC), condensed tannin content (CTC), antioxidant potential (FRAP, DPPH & CUPRAC), alpha amylase inhibition potential (AAIU) and alpha glucosidase inhibition potential (AGIU) in field pea determined by Pearson's correlation test.

	TPC	TFC	CTC	FRAP	DPPH	CUPRAC	AAIU	AGIU
TPC	1							
TFC	0.7	1						
CTC	0.86	0.8	1					
FRAP	0.89	0.82	0.96	1				
DPPH	0.83	0.62	0.8	0.83	1			
CUPRAC	0.95	0.72	0.89	0.91	0.86	1		
AAIU	0.17	0.04	0.05	0.07	0.25	0.28	1	
AGIU	0.52	0.38	0.27	0.28	0.42	0.57	0.24	1

Table 6. Relationship between total phenolic content (TPC), total flavonoid content (TFC), condensed tannin content (CTC), antioxidant potential (FRAP, DPPH & CUPRAC), alpha-amylase inhibition potential (AAIU) and alpha-glucosidase inhibition potential (AGIU) in cowpea determined by Pearson's correlation test.

	TPC	TFC	CTC	FRAP	DPPH	CUPRAC	AAIU	AGIU
TPC	1							
TFC	0.84	1						
CTC	0.83	0.82	1					
FRAP	0.97	0.88	0.80	1				
DPPH	0.82	0.98	0.79	0.87	1			
CUPRAC	0.84	0.98	0.81	0.88	0.99	1		
AAIU	-0.57	-0.47	-0.49	-0.63	-0.41	-0.40	1	
AGIU	0.59	0.70	0.48	0.57	0.66	0.62	-0.60	1

differences in the phenolic composition of field pea and cowpea cultivars showed that the cultivars with dark seed coat colour (brown and black) have higher protocatechuic acid and catechin hydrate content than cultivars with light seed coat colour.

α-amylase and α-glucosidase inhibitory activity

Retarding the absorption of glucose through the inhibition of carbohydrate metabolizing enzymes such as α-amylase and α-glucosidase is considered a therapeutic approach for the management of type II diabetes. The mild natural inhibitors of these enzymes are being preferred compared to synthetic inhibitors like acarbose and voglibose which often have gastrointestinal side effects (Stojkovic *et al*. 2019, Bischoff 1994, Kwon *et al*. 2007, Sreerama *et al*. 2012, Liu *et al*. 2016, Zhang *et al*. 2017). The inhibitory action of phenolics against α-amylase and α-glucosidase enzymes has been associated with their structure, which allows interaction with the enzyme or the substrate (Sun *et al*. 2019). The results from this *in-vitro* study showed that phenolic extracts of fieldpea and cowpea significantly inhibited the activity of pancreatic α-amylase and α-glucosidase enzymes (Table 4). The α-amylase inhibitory activity of field pea cultivars ranged from 19.3 to 36.3 α-amylase inhibition unit (AAIU)/mg seed and the strongest inhibition was shown by the cultivar Azad P3. The α-amylase inhibition potential of cowpea cultivars was comparable to field pea cultivars and it varied from 16.6 to 36.8 AAIU/mg seed) Pearson's correlation test showed that AAIU and TPC have a weak positive correlation (r = 0.16) in field peas but they are negatively correlated (r = -0.57) in cowpeas (Table 5 & 6).

The α-glucosidase inhibitory activity of field pea and cowpea cultivars ranged from 0.11 to 0.58 α-glucosidase inhibitory unit (AGIU)/mg seed and 0.4 to 1.97 AGIU/mg seed respectively (table 4). The field pea cultivar Arkel and the cowpea cultivar GC 901 showed the strongest inhibition of α-glucosidase. Contrary to AAIU values, the AGIU values showed a significant positive correlation with the TPC values in field pea (r = 0.51) as well as cowpea (r = 0.59) suggesting the role of phenolics in inhibiting the activity of α-glucosidase (Table 5 & 6).

Principle component analysis

The principal component analysis (PCA) biplot showed the relationship between primary variables and obtained principal components for field pea and cowpea datasets (Fig. 3a & 3b). The first two principal components accounted for 86.1% and 7.11% of the data variability, respectively, and together explained 93.21% of the total variability in field pea data. In the cowpea dataset, the first two principal components accounted for 89.8% and 5.55% of variability, respectively, and together explained for 95.35% of the total variability.

The results of the hierarchical cluster analysis are presented in Fig. 4. Two significant clusters were generated for field pea cultivars. The first cluster includes IPF 99-25, IPFD 1-10, IPF 5-19, P-489, IPFD 10-12, IPF 12-20, Arkel, EC-341792 and IPF 16-13. The second cluster has IPFD 2014-2, P-1586, Azad P-3, B-22, and EC 328758. The cowpea cultivars were also divided into two clusters. The first cluster includes TPTC 29, KBC 9, KBC 7, GC 901, and PL3. The second cluster includes TCS 160, RC 101, TC 901, GC 3, PL 2, DC 15, and DC 16.

CONCLUSION

The present study provides comparative insights into variations in phenolic content, phenolic profile, antioxidant and antidiabetic potential of field pea and cowpea cultivars. The cowpea cultivars showed higher phenolic content, stronger antioxidant potential, and a more diverse phenolic profile than the field pea cultivars. Also, the dark-seeded cultivars of these pulses showed higher antioxidant potential as compared to the light-seeded cultivars. The phenolic and antioxidant-rich field pea and cowpea cultivars identified here can be utilized in plant breeding and also in the development of novel functional foods. The phenolic extracts of these pulses also reduced the activity of α-amylase and α-glucosidase enzymes which suggests their efficacy in the management of type 2 diabetes. However, their effectiveness in reducing postprandial blood glucose levels needs

to be further validated in *in-vivo* models and also through epidemiological studies. Further research on the effect of specific phenolic acids and flavonoid compounds on the activity of carbohydrate metabolizing enzymes might lead to interesting insights in this research subject.

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AUTHORS CONTRIBUTIONS

Kalpana Tewari: project conceptualization, research experiments and writing; Vaibhav Kumar: project conceptualization, research experiments and writing; Ashok Kumar Parihar: provided experimental materials and writing; Sushmita Singh: writing and editing; Sudhir Kumar Jha: experiment; Namrata Laskar: writing; Neeraj Joshi: statistical analysis; Rishikesh Kumar: statistical analysis; Dharmendra Prasad Patel: writing and editing; M. Senthil Kumar: writing and editing; Girish Prasad Dixit: supervision and editing

COMPETING INTERESTS

The authors declare that there is no conflict of interest associated with this publication.

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