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Comparative evaluation of cowpea (*Vigna unguiculata* L.) genotypes for nutritional quality and antioxidant potential

Harveen Kaur*, Meenakshi Goyal and Devinder Pal Singh
Punjab Agricultural University, Ludhiana-141004, India
*Corresponding author e-mail: harveenbrar55@gmail.com
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Abstract
Ten cowpea genotypes were evaluated for better fodder quality, antioxidant and yield potentials. The average content of crude protein, neutral detergent fibre, acid detergent fibre, crude fibre, ash and ether extract was 16.63, 48.15, 36.10, 28.44, 10.54 and 2.91%, respectively. The average sugar and starch content was observed 20.45 and 32.31 mg/g, respectively. The phenol, tannin and flavonoid content was positively correlated with 2, 2-diphenyl-1-picryl hydrazyl free radical scavenging activity ($P<0.05$) and total reducing power ($P<0.01$). Saponin content was positively correlated with nitric oxide radical scavenging activity. Among all genotypes, UPC-628 genotype had low phenol and flavonoid content and C-88 had low tannin and saponin contents, respectively. The green fodder, dry matter and crude protein yields were found maximum in PFC-39, PFC-40 and PFC-12 genotypes, respectively. Nutritionally superior genotypes could be involved in breeding programme with high yielding genotypes PFC-39 and PFC-40 to develop nutritionally rich and high yielding genotypes.

Keywords: Antinutritional components, Antioxidant potential, Cowpea, Fodder quality, Fodder yield

Introduction
India is an agricultural country and approximately 3/4th population dwell in little more than 6 lakh villages. The total area under cultivation is about 169.7 million hectares. India has nearly 4.9% of total cultivated area under forages. Cowpea (*Vigna unguiculata* L.) is an important *Kharif* legume crop, belongs to the *Fabaceae* family. It is widely cultivated in the tropical and subtropical regions. In India, area under cowpea cultivation is 3.9 million hectares (Singh *et al.,* 2012). Cowpea contains maximum protein content and also rich in carbohydrates, fats, important minerals, vitamins, phenolic compounds, unsaturated fatty acids and antioxidants (Liyanage *et al.,* 2014). Nutrient balance is reliant on the nutritional quality of feed stuff and its digestibility. The protein quality of forage cowpea is negatively affected by the presence of antinutritional factors such as protease inhibitors, phytic acid, saponins, flavonoids, tannins and phenols. These antinutritional factors impair the digestibility of carbohydrates, proteins and minerals thereby limiting their nutrient utilization and decreasing the food quality (Kaur *et al.,* 2014). Tannins reduce the palatability of the feed and lower protein digestibility. Several cases of livestock death have been associated with high tannin content of some foliage (Bharathidhasan *et al.,* 2013).

Free radicals generated in several biochemical reactions are mediators of many diseases and cause structural and functional damage to protein, lipid, nucleic acid and cellular molecules (Kumaran and Karunakaran, 2007). Antioxidant components scavenge free radicals and reactive oxygen species thus cause inhibition of degenerative diseases (Zia-Ul-Haq *et al.,* 2012). Different methods have been used to measure antioxidant potential of cowpea, including 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power, total reducing power, nitric oxide and hydroxyl radical scavenging activity. The antioxidant capacity, antimutagenic activity and antiproliferative effects of legumes have been associated with the presence of phenolic compounds (Dong *et al.,* 2007). There is need to evaluate and identify genotype with high fodder yield and low antinutritional content so as to involve in breeding programme to develop better genotypes. Genotypes with maximum nutritional composition and antioxidant potential are helpful in increasing milk yield and lowering the risk of diseases caused by free radicals. Keeping this in mind the present investigation was aimed to evaluate different cowpea genotypes with respect to nutritional, antinutritional, antioxidant and yield potentials.

Materials and Methods
Experimental design: The present investigation was carried out on ten different cowpea genotypes.The CL-

367 and CL-88 are the released varieties and PFC-12, PFC-39 and PFC-40 are cowpea genotypes of Punjab Agricultural University, Ludhiana.The BL-1 and BL-2 are recommended varieties of Indian Grassland and Fodder Research Institute, Jhansi. The *Arka garima* genotype was developed by Central Coastal Agriculture Research Institute, Goa. The *Pusa sampada* and UPC-628 was developed by Indian Agricultural Research Institute, New Delhi and Gobind Ballah Pant University of Agricultural Technology, Pantnagar, respectively. Cowpea crop was raised in the experimental area of Forage Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana in July, 2016. The crop was planted in plots with 3.5 m bed length in three replications using randomized block design (RBD). Each plot consisted of 4 rows with row to row spacing 30 cm.

Sampling and laboratory analysis: For biochemical and quality analysis, five random plants from each plot were harvested at flowering stage and cut into small pieces. A 500 g sample was dried in a hot air oven at a temperature of 55^{o}-65^{o} C followed by processing in a grinding machine. Fodder samples were dried in hot air oven and then grinded for further analysis. The crude protein, crude fibre, ash content and ether extract were estimated as per AOAC (2005). Cell wall components (ADF and NDF) were estimated by Van soest *et al.* (1991). *In-vitro* dry matter digestibility content was estimated from protocol given by Tilley and Terry (1963). Total soluble sugars and starch content were estimated by Dubois *et al.* (1956). Standard curve was prepared by using glucose (10-100 µg) as a standard. Total phenols were estimated as per Swain and Hillis (1959). Tannins were estimated from protocol given by Sadasivam and Manickam (1992). Both phenol and tannin concentrations were expressed as tannic acid equivalents. Flavonoid was estimated as per Chang *et al.* (2002). The results were expressed as mg of rutin equivalents (RE/g). Saponins were estimated from the protocol given by Fenwick and Oakenfull (1983).

DPPH activity was determined by the method of Blois (1958). The percentage inhibition of reaction mixture was measured against reagent blank at 515 nm. Ferric reducing antioxidant power (FRAP) was determinedby the method of Benzie and Strain (1996). The FRAP activity was calculated from standard curve of iron sulphate heptahydrate (5–30 µg) run simultaneously. Total reducing power was determined by the method of Sreeramulu *et al.* (2009). The absorbance of reaction mixture was measured spectrophotometrically at 700 nm against reagent blank. Ascorbic acid (5–40 µg) was used as a standard. The hydroxyl ion radical scavenging activity was determined by the modified method of Li *et al.* (2008). Nitric oxide radical scavenging activity was estimated by the method of Marcocci *et al.* (1994). Free radical scavenging activity was calculated as follows:

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

Statistical analysis: Statistical analysis was performed by using SAS package (Version 9.3). Mean and standard deviation were calculated and Tukey's test was used to identify the significant differences among the genotypes. Pearson correlation test was conducted to determine correlation among variables. Significant levels were defined using ($P\leq0.05$).

Results and Discussion

Nutritional components: A significant ($P<0.05$) variation was observed between different cowpea genotypes with regard to quality traits (Table 1). Protein is an important trait in fodders as it is required for the growth, development and production of ruminant animals. The crude protein content (%) was ranged from 13.53 to 20.66 with an average value of 16.63. The results were in accordance with previous studies on cowpea fodder (Devasena *et al*., 2009; Sallam and Ibrahim, 2016). The protein content of other leguminous forages in *Rabi* season varied from 18.5 to 21.8% (Goyal *et al*., 2017). Generally high protein forages are more digestible and provide more energy. The *in-vitro* dry matter digestibility (IVDMD) was found positively correlated with crude protein ($P<0.01$, r= 0.908**) (Table 5) and found maximum in PFC-12 genotype. Acid detergent fibre and neutral detergent fibre are major indicators of digestibility and negatively affects feed quality (Eskandari *et al.,* 2009). The ADF and NDF content was positively related with each other ($P<0.01$, r= 0.862**). The ADF and NDF content (%) varied significantly from 27.10 to 41.83 and 38.3 to 52.4, respectively. The minimum ADF and NDF content was exhibited by *Pusa sampada* genotype. Our results were in accordance with previous studies on ADF content in cowpea (Devasena *et al.,* 2009; Dahmardeh *et al.,* 2009) and NDF content in cowpea (Prusty *et al.,* 2013). The crude fibre (CF) content was found maximum in UPC-628 and negatively correlated with IVDMD content ($P<0.05$). The average content of ash and ether extract was found to be 10.54 and 2.91%, respectively. The ash content of ten different cowpea fodder genotypes varied from 9.97 to 12.20% (Mahala *et al.,* 2014). The average sugar and starch content was observed 20.45 and 32.31 mg/g, respectively. Among all genotypes, *Pusa sampada*

Table 1. Study of nutritional composition of different fodder cowpea genotypes

Genotypes	Crude protein (%)	IVDMD (%)	Acid detergent fibre (%)	Neutral detergent fibre (%)	Crude fibre (%)	Ash (%)	Ether extract (%)	Total soluble sugars (mg/g)	Starch content (mg/g)
BL-1	16.10 ± 0.18^{de}	72.10 ± 0.83^{cd}	33.29 ± 0.72^{f}	44.85 ± 0.69^{e}	25.83 ± 0.61^{e}	11.39 ± 0.17^{ab}	3.59 ± 0.15^{b}	16.43 ± 0.60^{c}	37.67 ± 0.75^{ab}
BL-2	16.71 ± 0.36^{cd}	73.41 ± 0.49^{bc}	37.66 ± 0.26^{d}	49.21 ± 0.58^{c}	29.66 ± 1.66^{c}	11.79 ± 0.20^{a}	2.21 ± 0.10^{f}	13.37 ± 1.06^{de}	39.95 ± 1.34^{a}
C-88	16.01 ± 0.17^{c}	70.44 ± 0.59^{d}	40.61 ± 0.20^{b}	50.15 ± 0.54^{bc}	33.62 ± 0.36^{ab}	9.91 ± 0.23^{ef}	3.01 ± 0.03^{d}	12.55 ± 0.28^{de}	25.60 ± 0.96^{cd}
CL-367	17.52 ± 0.36^{cd}	71.56 ± 0.51^{d}	39.53 ± 0.24^{c}	50.72 ± 0.42^{bc}	32.60 ± 0.76^{bc}	10.13 ± 0.17^{def}	2.76 ± 0.10^{d}	14.20 ± 0.27^{d}	35.72 ± 0.97^{b}
PFC-40	15.54 ± 0.36^{e}	67.21 ± 0.60^{e}	37.70 ± 0.34^{d}	47.06 ± 0.49^{d}	27.32 ± 0.93^{d}	10.46 ± 0.23^{cde}	4.15 ± 0.06^{a}	17.71 ± 0.67^{c}	35.66 ± 1.24^{b}
PFC-39	14.55 ± 0.25^{f}	65.62 ± 0.98^{e}	37.35 ± 0.33^{d}	51.02 ± 0.98^{ab}	28.51 ± 0.38^{cd}	9.76 ± 0.21^{f}	2.43 ± 0.09^{ef}	13.10 ± 0.30^{de}	33.92 ± 2.70^{b}
Arka garima	19.23 ± 0.23^{b}	74.13 ± 0.83^{ab}	30.25 ± 0.29^{g}	46.73 ± 0.26^{d}	21.23 ± 0.64^{g}	10.59 ± 0.20^{cd}	3.03 ± 0.05^{cd}	40.88 ± 0.87^{b}	29.24 ± 1.58^{c}
PFC-12	20.66 ± 0.28^{a}	75.82 ± 0.92^{a}	35.66 ± 0.30^{e}	50.92 ± 0.85^{ab}	27.41 ± 1.06^{d}	10.69 ± 0.14^{cd}	1.87 ± 0.09^{g}	16.67 ± 0.35^{c}	27.70 ± 0.68^{c}
Pusa sampada	16.47 ± 0.18^{cd}	62.79 ± 0.40^{f}	27.10 ± 0.22^{h}	38.38 ± 0.35^{f}	23.53 ± 1.05^{f}	11.01 ± 0.29^{bc}	3.32 ± 0.11^{bc}	47.73 ± 0.66^{a}	34.01 ± 1.47^{b}
UPC-628	13.53 ± 0.22^{g}	59.81 ± 0.92^{g}	41.83 ± 0.18^{a}	52.43 ± 0.34^{a}	34.69 ± 0.60^{a}	9.68 ± 0.12^{f}	2.74 ± 0.18^{de}	11.87 ± 0.84^{e}	23.69 ± 0.36^{d}
Mean	16.63	69.29	36.10	48.15	28.44	10.54	2.91	20.45	32.31

Values are mean ± SD of triplicates

Values with different letters in the same column are significantly different (P<0.05)

genotype exhibited maximum sugar content followed by *Arka garima* genotype. Starch content was found maximum in BL-2 which was statistically at par with BL-1 genotype.

Antinutritional components/factors: A significant (P<0.05) difference was reported among fodder genotypes for antinutritional factors (Table 2). Total phenols (mg/g) ranged from 1.55 to 3.73 with an average value of 2.29. The maximum phenol content was observed in *Pusa sampada* genotype which was statistically at par with *Arka garima* genotype. Genotypes with low phenolic content are preferred for nutritional purpose, as they are known to decrease the digestibility of proteins, minerals and carbohydrates (Sahoo *et al.,* 2016) and also lower the activity of digestive enzymes (Othman *et al.,* 2007). The genotypes with high phenols are beneficial to crops against insect/pest resistance and also as a source of bioactive compounds (Xu and Chang, 2008). Tannin content (mg/g) among different forage genotypes was varied from 1.04 to 2.37 with mean value of 1.59. The concentration of condensed tannins above 4 per cent is toxic for ruminants as they are harmful to a variety of rumen microbes (Waghorn *et al.,* 1994). In our study, total tannin content of cowpea genotypes was much lesser than 4% so may be considered nutritionally good for livestock consumption. Total flavonoid content (mg/g) revealed significant variation from 2.91 to 6.68 with mean value of 4.18. The maximum flavonoid content was found in *Pusa sampada* and minimum in UPC-628. The accumulation of flavonoids in external cells acts as UV-screen and protects the plant from harmful radiations (Agati *et al.,* 2012). Flavonoids combat oxidative stress in plants by quenching and inhibiting the generation of reactive oxygen species. They also act as protectants of plants from insect and pest infestations by influencing their behavior, growth and development (Harbourne, 1993). The phenol, tannin and flavonoid content was found to be positively correlated with DPPH free radical scavenging activity (P<0.05) and total reducing power (P<0.01) (Table 6). Saponin content (mg/g) was ranged from 8.52 to 11.84 and found maximum in PFC-40 genotype which was at par with BL-1 genotype. Saponins also have lytic action on erythrocyte membrane when consumed in larger amounts. Despite of this, they lower harmful LDL-cholesterol level and stimulate immune response (Segal *et al.,* 2003).

Antioxidant potential: 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) scavenging assay is extensively used to test the ability of compounds to act as free radical scavenger of

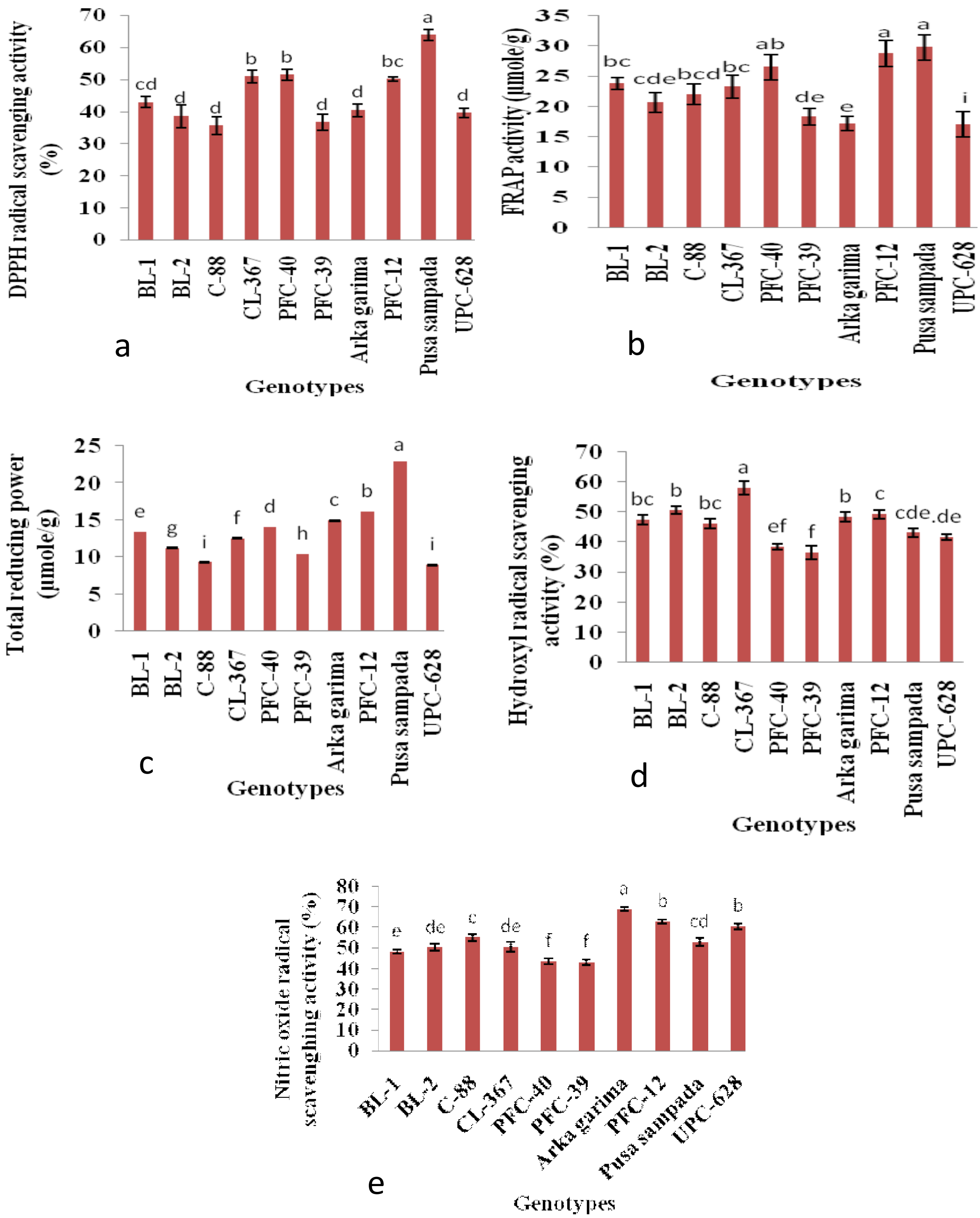

Fig 1. Evaluation of (a) DPPH free radical scavenging activity, (b) total reducing power, (c) FRAP activity, (d) hydroxyl radical scavenging activity and (e) nitric oxide radical scavenging activity in fodder cowpea genotypes. Vertical bars show mean ± SD of triplicates. Values with different letters are significantly different (P<0.05)

hydrogen donors present in fodder extract. In cowpea fodder, DPPH scavenging activity (%) revealed significant variation from 35.54 to 63.81 with an average value of 44.97 (Fig 1). The maximum DPPH scavenging activity was observed in *Pusa sampada* genotype. FRAP activity (mg/g) among different cowpea genotypes varied from 17.03 to 29.74 with an average value of 22.69. The maximum FRAP activity was found in *Pusa sampada* genotype which was at par with PFC-12 and PFC-40 genotypes. Legumes manifest remarkable antioxidant activity (Sinha *et al.,* 2013) and serve as an excellent dietary source of natural antioxidants for prevention of

Table 2. Study of antinutritional factors of different fodder cowpea genotypes

Genotypes	Total phenols (mg/g)	Tannins(mg/g)	Flavonoid(mg/g)	Saponin(mg/g)
BL-1	$2.33{\pm}0.10^{bcd}$	$1.54{\pm}0.13^{b}$	$4.11{\pm}0.22^{cd}$	$11.24{\pm}0.13^{ab}$
BL-2	$1.61{\pm}0.13^{e}$	$1.20{\pm}0.08^{c}$	$3.28{\pm}0.03^{de}$	$8.59{\pm}0.54^{f}$
C-88	$1.58{\pm}0.28^{e}$	$1.04{\pm}0.12^{c}$	$3.00{\pm}0.42^{e}$	$8.52{\pm}0.15^{f}$
CL-367	$2.44{\pm}0.27^{bc}$	$1.66{\pm}0.14^{b}$	$4.03{\pm}0.14^{cd}$	$9.03{\pm}0.32^{f}$
PFC-40	$2.49{\pm}0.30^{b}$	$1.78{\pm}0.10^{b}$	$4.22{\pm}0.04^{c}$	$11.84{\pm}0.16^{a}$
PFC-39	$1.88{\pm}0.14^{de}$	$1.52{\pm}0.12^{b}$	$3.78{\pm}0.26^{cde}$	$10.88{\pm}0.15^{bc}$
Arka garima	$3.38{\pm}0.30^{a}$	$2.20{\pm}0.13^{a}$	$5.77{\pm}0.14^{b}$	$10.05{\pm}0.20^{de}$
PFC-12	$1.92{\pm}0.22^{cde}$	$1.53{\pm}0.15^{b}$	$4.07{\pm}0.25^{cd}$	$10.52{\pm}0.34^{cd}$
Pusa sampada	$3.73{\pm}0.14^{a}$	$2.37{\pm}0.12^{a}$	$6.68{\pm}0.40^{a}$	$9.81{\pm}0.09^{e}$
UPC-628	$1.55{\pm}0.07^{e}$	$1.10{\pm}0.05^{c}$	$2.91{\pm}0.03^{e}$	$10.10{\pm}0.18^{de}$
Mean	2.29	1.59	4.18	10.06

Values are mean ± SD of triplicates; Values with different letters in the same column are significantly different (P<0.05)

Table 3. Study of yield parameters of different fodder cowpea genotypes

Genotypes	Plant height (cm)	Vine length (cm)	Number of leaves per plant	Leaf/stem ratio	Dry matter (%)
BL-1	$54.3{\pm}2.08^{cd}$	$124.3{\pm}6.02^{abc}$	$69.3{\pm}4.04^{ef}$	$0.88{\pm}0.01^{b}$	$13.0{\pm}0.23^{f}$
BL-2	$61.3{\pm}1.15^{c}$	$124.6{\pm}17.89^{abc}$	$85.6{\pm}5.50^{cde}$	$1.11{\pm}0.01^{a}$	$14.4{\pm}0.15^{e}$
C-88	$73.0{\pm}1.00^{a}$	$136.6{\pm}5.77^{a}$	$43.3{\pm}7.63^{g}$	$0.36{\pm}0.02^{h}$	$14.1{\pm}0.30^{e}$
CL-367	$59.3{\pm}1.15^{c}$	$140.0{\pm}8.18^{a}$	$93.3{\pm}9.45^{cd}$	$0.72{\pm}0.02^{c}$	$15.5{\pm}0.10^{d}$
PFC-40	$71.3{\pm}1.15^{b}$	$146.3{\pm}10.69^{a}$	$66.3{\pm}9.07^{ef}$	$0.43{\pm}0.01^{g}$	$16.0{\pm}0.20^{c}$
PFC-39	$61.0{\pm}1.00^{c}$	$100.6{\pm}9.01^{cd}$	$75.6{\pm}11.01^{def}$	$0.61{\pm}0.02^{d}$	$14.6{\pm}0.26^{e}$
Arka garima	$71.3{\pm}1.52^{b}$	$103.0{\pm}8.18^{cd}$	$116.7{\pm}6.50^{b}$	$0.71{\pm}0.02^{c}$	$16.1{\pm}0.10^{c}$
PFC-12	$77.0{\pm}1.00^{a}$	$106.6{\pm}12.58^{bcd}$	$218.3{\pm}7.63^{a}$	$0.52{\pm}0.01^{e}$	$16.6{\pm}0.15^{b}$
Pusa sampada	$72.3{\pm}2.51^{b}$	$79.3{\pm}3.78^{d}$	$103.6{\pm}5.50^{bc}$	$0.49{\pm}0.02^{f}$	$17.2{\pm}0.15^{a}$
UPC-628	$71.3{\pm}1.15^{b}$	$133.3{\pm}11.5^{ab}$	$61.0{\pm}6.55^{fg}$	$0.40{\pm}0.01^{g}$	$15.2{\pm}0.10^{d}$
Mean	67.2	119.5	93.3	0.62	15.2

Values are represented as mean ± standard deviation of triplicates; Values with different letters in the same column are significantly different (P<0.05)

Table 4. Yield data of different fodder cowpea genotypes

Genotypes	Green fodder yield (q/ha)	Dry matter yield (q/ha)	Crude protein yield (q/ha)
BL-1	$281.7{\pm}17.24^{ab}$	$36.7{\pm}2.24^{ab}$	$5.9{\pm}0.36^{bc}$
BL-2	$253.9{\pm}47.42^{ab}$	$36.7{\pm}6.85^{ab}$	$6.1{\pm}1.14^{abc}$
C-88	$257.9{\pm}24.41^{ab}$	$36.3{\pm}3.44^{ab}$	$6.2{\pm}0.60^{abc}$
CL-367	$269.8{\pm}7.23^{ab}$	$41.8{\pm}1.21^{a}$	$6.9{\pm}0.18^{ab}$
PFC-40	$285.7{\pm}5.50^{ab}$	$45.8{\pm}0.88^{a}$	$7.1{\pm}0.14^{ab}$
PFC-39	$309.5{\pm}13.01^{a}$	$45.1{\pm}1.89^{a}$	$6.6{\pm}0.28^{ab}$
Arka garima	$47.6{\pm}12.05^{d}$	$7.6{\pm}1.93^{c}$	$1.5{\pm}0.33^{d}$
PFC-12	$230.2{\pm}24.97^{bc}$	$38.2{\pm}4.15^{ab}$	$7.9{\pm}0.77^{a}$
Pusa sampada	$166.7{\pm}24^{c}$	$28.7{\pm}4.14^{b}$	$4.7{\pm}0.68^{c}$
UPC-628	$265.8{\pm}17.89^{ab}$	$40.4{\pm}2.72^{a}$	$5.5{\pm}0.36^{bc}$
Mean	236.88	35.7	5.8

Values are represented as mean ± standard deviation of triplicates; Values with different letters in the same column are significantly different (P<0.05)

Table 5. Correlation coefficient between nutritional components and yield of fodder cowpea

	Crude protein	Crude fibre	Ether Extract	Ash	Nitrogen free extract	Neutral detergent fibre	Acid detergent fibre	*In vitro* dry matter digestibility	Soluble sugars	Starch	Green fodder yield
Crude fibre	-0.779**										
Ether Extract	-0.263	0.156									
Ash Content	0.451	0.022	0.039								
Nitrogen free extract	0.412	-0.869**	-0.254	-0.439							
Neutral detergent fibre	-0.176	-0.019	-0.545	-0.527	0.335						
Acid detergent fibre	-0.369	0.273	-0.234	-0.519	0.008	0.862**					
In vitro dry matter digestibility	0.908**	-0.653*	-0.205	0.559	0.297	-0.036	-0.234				
Soluble sugars	0.268	-0.220	0.278	0.269	-0.007	-0.806**	-0.899**	0.071			
Starch	0.044	0.355	0.216	0.662*	-0.623	-0.370	-0.259	0.193	0.016		
Green fodder yield	-0.432	0.423	-0.017	-0.197	-0.237	0.421	0.679*	-0.349	-0.828**	0.230	
Dry matter yield	-0.396	0.356	-0.037	-0.248	-0.179	0.401	0.655*	-0.393	-0.757*	0.186	0.966**

*Significant at 5% level, **Significant at 1% level

various diseases and health promotion. Total reducing power of different cowpea fodder genotypes varied significantly from 8.95 to 22.95 µmole/g. The maximum total reducing power was found in *Pusa sampada* and minimum in UPC-628 genotype. The hydroxyl radical scavenging rate (%) among cowpea genotypes revealed significant variation from 36.47 to 57.95 with an average value 45.91. The maximum hydroxyl radical scavenging capacity was observed in CL-367 and minimum in PFC-40 genotype. Hydroxyl radical scavenging capacity of a plant extract is directly related to its antioxidant activity (Babu *et al.,* 2001). Hydroxyl radical reacts with lipid, polypeptides, proteins and DNA (Manian *et al.,* 2008). The nitric oxide radical scavenging rate (%) among different cowpea fodder genotypes varied significantly from 42.98 to 69.02 with an average value of 53.60. The maximum nitric oxide radical scavenging activity was found in *Arka garima* genotype and minimum in PFC-39 genotype. In a previous study, 72.48 % and 70.43% DPPH and nitric oxide radical scavenging activity was observed in leaves extract of *Leonotis leonurus* (L.), respectively.

Nitric oxide is widespread signaling molecule and participates in some cellular functions of the body. It also acts as a neurotransmitter and an important mediator of the immune response (Fang *et al.,* 2002). Nitric oxide radical scavenging activity showed positive correlation with saponin ($P<0.05$) (Table 6).

Yield parameters: The increase in the yield was mainly due to increasing plant height, stem diameter, number of leaves, leaf area per plant and number of branching per plant (Hasan *et al.,* 2010). Plant height and vine length of cowpea genotypes varied from 54.3 to 77.0 cm and 79.3 to 146.3 cm, respectively (Table 3). Our results on plant height were in agreement with that reported previously for cowpea (Hassan *et al.*, 2010; Kumar and Pandita, 2016). Number of leaves varied significantly and ranged between 43.3-218.3. The variation in performance of the studied cultivars depends on the genetic and environmental factors (Nwosu *et al.,* 2013). Leaf/stem ratio is an important component of determining forage quality. Young plants have high leaf/stem ratio as compared to mature plants. It was varied significantly from 0.36 to 1.11. Our results showed good agreement with previous studies on cowpea leaf/stem ratio (Dhonde *et al.,* 2016; Shekara *et al.,* 2012). Dry matter contnt varied significantly from 13.0 to 17.2%. Dry matter percentage was observed maximum in *Pusa sampada* genotype and minimum in BL-1 genotype. The results were in accordance with the findings of Devasena *et al.* (2009).

Table 6. Correlation coefficients between antinutritional factors and antioxidant potential of fodder cowpea

	Phenol	Tannin	Flavonoid	Saponin	DPPH	FRAP	TRP	HRSA
Tannin	0.974**							
Flavonoid	0.974**	0.973**						
Saponin	0.189	0.301	0.185					
DPPH	0.674*	0.691*	0.690*	0.195				
FRAP	0.342	0.372	0.402	0.190	0.823**			
TRP	0.831**	0.847**	0.901**	0.191	0.873**	0.727*		
HRSA	0.034	-0.029	-0.014	-0.579	0.08	0.09	0.02	
NORSA	0.172	0.124	0.204	0.764*	-0.077	-0.192	0.125	0.326

*Significant at 5% level; **Significant at 1% level; TRP: Total reducing power; HRSA: Hydroxyl radical scavenging activity; NORSA: Nitric oxide radical scavenging activity

Yield: The green fodder yield (q/ha) of cowpea genotypes revealed significant variation, ranging from 47.6 to 309.5 with an average value of 236.88 (Table 4). The maximum yield was observed in PFC-39 and minimum was in *Arka garima* genotype. Our results were in accordance with previous studies on cowpea green fodder yield (Pal *et al.,* 2014; Sharma *et al.,* 2008; Surve *et al.,* 2012). The dry fodder yield (q/ha) was varied from 28.77 to 45.88 with an average value of 35.77. The maximum dry fodder yield was observed in PFC-40. The maximum crude protein yield was observed in PFC-12 and minimum in *Arka garima.* The DMY and CPY was reported to vary from 30.65-43.40 q/ha and 4.40-6.41q/ha, respectively in cowpea genotypes (Shekara *et al*., 2012).

Conclusion

The PFC-12 was considered nutritionally important as it possessed maximum crude protein, *in-vitro* dry matter digestibility, crude protein yield, plant height, leafiness and medium antinutritional factors. The green fodder and dry matter yield was found maximum in PFC-39 and PFC-40 genotypes, respectively. The genotype *Pusa sampada* had possessed maximum DPPH scavenging activity, FRAP activity and total reducing power. High antioxidant activity contributes significantly to the prevention of degenerative diseases associated with free radical damage. The cowpea genotypes with high nutritional value (PFC-12) and high antioxidant potential (*Pusa sampada*) could be involved in breeding programme with high yielding genotypes (PFC-39, PFC-40) for the production of superior genotypes.

References

Agati, G., E. Azzarello, S. Pollasrtri and M. Tattini. 2012. Flavonoids as antioxidants in plants: location and functional significance. *Plant Science* 196: 67-76.

AOAC. 2005. *Official Methods of Analysis*. 18th ed. Association of Official Analytical Chemists. Maryland, U.S.A.

Babu, B. H., B. S. Shylesh and J. Padikkala. 2001. Antioxidant and hepato-protective effect of *Alanthus icici focus*. *Fitoterapia* 72: 272-277.

Benzie, I. F. and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry* 239: 70-76.

Bharathidhasan, A., K. Viswanathan and V. Balakrishnan. 2013.Total phenolics, non-tannin phenolics and total tannin content of commonly available forages for ruminants in Tamil Nadu. *Range Management and Agroforestry* 34: 205-208.

Blois, M. S. 1958. Antioxidant determination by the use of stable free radical. *Nature* 181: 1199-1200.

Chang, C., M. Yang, H. Wen and J. Chem. 2002. Estimation of total flavonoid content in *Propolis* by two complementary colorimetric methods. *Journal of Food and Drug Analysis* 10: 178-182.

Dahmardeh, M., A. Ghanbari, B. Syasar and M. Ramroudi. 2009. Effect of intercropping maize (*Zea mays* L.) with cowpea (*Vigna unguiculata* L.) as green forage yield and quality evaluation. *Asian Journal of Plant Science* 8: 235-239.

Devasena, B., A. Ravi and R. J. Prasad. 2009. Study on nutrient composition and yield of cowpea (*Vigna sinensis*) varieties. *Indian Journal of Animal Nutrition* 26: 251-254.

Dhonde, A. S., M. S. Pilane and A. N. Mahatre. 2016. Effect of intercropping of maize (*Zea mays* L.) + cowpea (*Vigna unguiculata* L.) on leaf stem ratio, maize equivalent yield and land equivalent ratio. *Journal of Agroecology and Natural Resource Management* 3: 27-29.

Dong, M., X. He and R. H. Liu. 2007. Phytochemicals of black bean and seed coats: isolation, structure elucidation and their anti-proliferative and antioxidant activities. *Journal of Agricultural and Food Chemistry* 55: 6044-6051.

Dubois, M., K. N. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith.1956. Colorimetric method for the determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.

Eskandari, H., A. Ghanbari and A. Javanmard. 2009. Intercropping of cereals and legumes for forage production. *Notulae Scientia Biologicae* 1: 07-13.

Fang, Y. Z., S. Yang and G. Wu. 2002. Free radicals, antioxidants and nutrition. *Journal of Nutrition* 18: 872-879.

Fenwick, D. E. and D. Oakenfull. 1983. Saponin content of food plants and some prepared foods. *Journal of the Science of Food and Agriculture* 34: 186-191.

Goyal, M., H. Kaur, D. P. Singh and U.S. Tiwana. 2017. Evaluation of nutritional quality and yield of winter forages prevalent in Punjab. *Range Management and Agroforestry* 38: 249-253.

Harbourne, J. B. 1993. *The Flavonoids: Advances in Research*. Chapman and Hall, London.

Hasan, M. R., M. A. Akbar, Z. H. Khandaker and M. M. Rahman. 2010. Effect of nitrogen fertilizer on yield contributing character, biomass, yield and nutritive value of cowpea forage. *Bangladesh Journal of Animal Science* 39: 83-88.

Kaur, S., A. K. Gupta, K. Narinder and M. Javed. 2014. Biochemical and nutritional characterization of chickpea (*Cicer arietinum*) cultivars. *Indian Journal of Agricultural Science* 84: 479-486.

Kumaran, A. and R. J. Karunakaran. 2007. *In-vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT Science Direct* 40: 344-352.

Kumar, A. and V. K. Pandita. 2016. Effect of integrated nutrient management on seed yield and quality in cowpea. *Legume Research* 39: 448-452.

Li, Y., B. Jiang, T. Zhang, Z. Mu and J. Liu. 2008. Antioxidant and free radical scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry* 106: 44-450.

Liyanage, R., O. S. Perera and R. Sivakanesan. 2014. Nutritional and antioxidant content of commonly consumed cowpea cultivars in Sri Lanka. *Journal of Food Legumes* 27: 215-217.

Mahala, A. G., A. A. Elussein, S. M. Gasim and A. A. Abdelmulla. 2014. Nutritive values of eight genotypes of cowpea whole plants as fodder. *Journalof Veterinary Medicine and Animal Production* 5: 42-50.

Manian, R., N. Anusuya, P. Siddhuraju and S. Manian. 2008. The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.), *Ficus benghalenis* L. and *Ficus racemosa* L. *Food Chemistry* 107: 1000-1007.

Marcocci, L., J. J. Maguire, M. T. Droy-Lefaix and L. Packer. 1994. The nitric oxide scavenging property of *Ginkgo biloba* extract EGB 761. *Biochemical and Biophysical Research Communications* 201: 748-755.

Nwosu, D. J., B. D. Olatunbosun and I. S. Adetiloye. 2013. Genetic variability, heritability and genetic advance in cowpea genotypes in two agro-ecological environments. *Greener Journal of Biological Sciences* 3: 202-207.

Othman, A., A. Ismail, N. A. Ghani and I. Adenan.2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chemistry* 100: 1523-1530.

Pal, M. S., A. Reza, Y. P. Joshi and U. B. S. Panwar. 2014. Production potential of forage sorghum (*Sorghum bicolor* L.) under different intercropping systems. *Agriculture and Sustainable Development* 2: 87-91.

Prusty, S., S. S. Kundu, V. Kumar and C. Datt. 2013. Dry matter and neutral detergent fiber degradation kinetics of roughages in relation to carbohydrate and protein fractions. *Indian Journal of Animal Nutrition* 30: 374-380.

Sadasivam, S. and A. Manickam.1992. Phenolics. In: *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Ltd, New Delhi, India. pp.187-188.

Sahoo, B., A. K. Garg, R. K. Mohanta, R. Bhar, P. Thirumurugan, A. K. Sharma and A. B. Pandey. 2016. Nutritional value and tannin profile of forest foliages in temperate sub-Himalyas. *Range Management and Agroforestry* 37: 228-232.

Sallam, A. M. and H. I. M. Ibrahim. 2016. Morphological, physiological and chemical traits of some forage cowpea genotypes.*American-Eurasian Journal of Agricultural and Environmental Science* 16: 302-311.

Segal, R., I. Milo-Goldzweig, H. Schupper and D. V. Zaitschek. 2003. Effect of ester groups on the hemolytic action of saponins. *Pharmacology* 19: 2501-2507.

Sharma, R. P., A. K. Singh, B. K. Poddar and K. R. Raman. 2008. Forage production potential and economics of maize (*Zea mays*) with legumes intercropping under various row proportions. *Indian Journal of Agronomy* 53: 121-124.

Shekara, B. G., B. S. Sowmyalatha and C. Baratkumar. 2012. Effect of phosphorus levels on forage yield of fodder cowpea. *Journal of Horticulture Letters* 2: 325-326.

Singh, A. K., B. P. Bhatt, P. K. Sundaram, S. Kumar, R. C. Bharati, N. Chandra and M. Rai. 2012. Study of site specific nutrients management of cowpea seed production and their effect on soil nutrient status. *Journal of Agriculture Science* 4: 191-198.

Sinha, S.K., M. Kumar, A. Kumar, S. Bharti and V.K. Shahi. 2013. Antioxidant activities of different tissue extract of faba bean (*Vicia faba* L.) containing phenolic compounds. *Legume Researc*h 36: 496-504.

Sreeramulu, D., C. V. K. Reddy and M. Raghunath. 2009. Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. *Indian Journal of Biochemistry and Biophysics* 46: 112-115.

Surve, V. H., P. R. Patil and M. K. Arvadia. 2012. Performance of fodder based intercropping of sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.) and cowpea (*Vigna unguiculata* L.) under different row ratio. *Agricultural Science Digest* 32 : 336-39.

Swain, T. and E. Hillis.1959. The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture* 10: 3-8.

Tilley, J. M. A. and R. A. Terry. 1963. A two stage technique for the in vitro digestion of force crops. *British Journal of Grassland Society* 18: 104-111.

Van soest, P. J., J. B. Robertson and B. A. Lewis.1991. Methods for dietary fibre neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74: 3583-3597.

Waghorn, G. C., L. D. Shelton and W. C. Mc Nabb. 1994. The effect of condensed tannin in *Lotus pedunculatus* on nutritive value for sheep. Nitrogenous aspects. *Journal of Agriculture Science* 123: 109-119.

Xu, B. and S. K. Chang. 2008. Antioxidant capacity of seed coat, dehulled bean and whole black soybeans in relation to their distribution of total phenolic acids, anthocyanins and isoflavones. *Journal of Agriculture and Food Chemistry* 56: 8365-8373.

Zia-Ul-Haq, M., S. Ahmad, L. Calani, T.Mazzeo, D. Del Rio, N. Pellegrini and F. V. De. 2012. Compositional study and antioxidant potential of *Ipomoea herderacea* Jacq. and *Lepidium sativum* L. seeds. *Molecules* 17: 10306-10321.