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Antioxidant Activity Potential of Selected Genotypes of Cowpea (*Vigna unguiculata*)

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ABSTRACT

Legume seeds are rich in many nutrient components including protein, starch, dietary fibre, certain fatty acids and micronutrients (vitamins, trace minerals). They are also a rich source of many bioactive non-nutrient compounds including phenolic antioxidants. These antioxidants exhibit a wide range of physiological health promoting properties, such as anti-allergenic, anti-inflammatory, anti-atherogenic, anti-microbial, anti-carcinogenic, cardio-protective and vasodilatory effects. Legume seed coats are rich sources of polyphenolics and natural antioxidants that could replace the synthetic antioxidants in foods. Legumes are decorticated to remove the outer fibrous layer (seed coat) to yield the split cotyledon (endosperm). This milling process significantly changes the phytochemical potential of legumes. In the present study, antioxidant properties of morphological fractions of four improved cowpea genotypes for high yield and early maturity viz. Pant Lobia-2 (PL-2), Pant Grain Cowpea-11 (PGCP-11), Pant Grain Cowpea-12 (PGCP-12), and Pant Grain Cowpea-13 (PGCP-13) were evaluated. The seed samples were dehulled into two fractions i.e. seed coat and cotyledon manually. Total phenol content (TPC) and antioxidant activity was measured by DPPH method using three replicates. The fraction of seed coat varied from 12.22±0.02 (PGCP-13) to 13.91±0.02 g (PGCP-11) whereas cotyledon varied from 86.07±0.03 (PGCP-13) to 87.76±0.02 g (PGCP-11). TPC was highest in PGCP-11 (seed coat: 455.64 mg GAE/ 100g; cotyledon: 258.81 mg GAE/ 100g) and least in PGCP-13 (seed coat: 254.68 mg GAE/ 100g; cotyledon: 163.25 mg GAE/ 100g) on dry weight basis. Similarly, antioxidant activity was highest in PGCP-11 (seed coat: 82.3%; cotyledon: 43.3%) and least in PGCP-13 (seed coat: 32.3%; cotyledon: 15.1%) on dry weight basis. Cowpea genotypes differed significantly with respect to their TPC and antioxidant activity. Seed coat had higher TPC as well as antioxidant activity than the cotyledon fraction of all the cowpea genotypes under study. The antioxidant properties of the seed coat in all the genotypes were manifold higher as compared to the whole legume or its respective cotyledon and removal of seed coat significant reduced the antioxidant activity.

Keywords: *Cowpea, Seed coat, Cotyledons, Antioxidant activity, Total phenol content*

INTRODUCTION

Oxygen being an essential element for life has a role in performing various biological functions of the body such as catabolism of fat, protein and carbohydrates in order to generate energy for growth and other activities. However, a contravening aspect of oxygen as a toxic agent has also been sighted.

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Though, it is not dangerous by itself but is involved in the generation of various kind of free reactive oxygen species (ROS). ROS formed through the metabolism or through the action of ionizing radiation, can interact with the bio-molecules and ultimately lead to onset of various degenerative diseases such as cancer, cardiovascular diseases, radiation damage and other illnesses (Lobo et al., 2010).

Cowpea (*Vigna unguiculata*) which is also known as blackeye pea, southern pea, and crowder pea, used mainly as a grain crop has potential antioxidants present in both, its seed coats and cotyledons. Also, phenols, which are a part of antioxidants, have much favourable effect on human health. They decrease the risk of heart disease by inhibiting the low density lipoprotein (LDL). A large range of low and high molecular weight phenols exhibiting antioxidant properties have been studied and proposed to be used as antioxidants against lipid oxidation. Data on the phenolic composition of cowpea seed coats have been limited and restricted to a few varieties. In the previous study of Oboh (2006), the presence of phenolic compounds was reported in the seed coats of five differently coloured varieties of cowpea. Cowpea seeds are increasingly consumed as human food nowadays, but the beneficial effects of their bioactive compounds remain largely unexplored. The evaluation of antioxidant potential in these cowpea seed coats and cotyledons might be a fruitful approach for advocating them as functional food also, in addition to being a potential protein and carbohydrate sources. In the present study, an attempt has been made to study antioxidant potential including total phenol content in both seed coat and cotyledons in one released variety Pant Lobia-2 and three breeding lines in process of recognition viz., PGCP-11, PGCP-12 and PGCP-13.

MATERIALS AND METHODS

Legume samples

To protect against destructive nature of free radicals, nature has created an antioxidant defence system composed of a group of compounds and enzymes potent enough to remove free radicals before they cause tissue damage. However supplementing the natural defence mechanism with dietary antioxidants might offer better protection against ROS induced oxidative damage (Oboh, 2006).

Four cowpea varieties namely, Pant Lobia-2 (PL-2) (released in the year 2008 by SVRC Uttarakhand), and breeding lines (Pant Grain Cowpea-11 (PGCP-11), Pant Grain Cowpea-12 (PGCP-12), Pant Grain Cowpea-13 (PGCP-13)) (Figure 1) were obtained from Breeder's Seed Production Centre, Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar. Samples from each replication (500 g) were chosen individually and randomly.

Dehulling of grains

Freshly harvested whole raw cowpea seeds of different varieties under study were at first conditioned by keeping at room temperature for 4 hours. The purpose of conditioning step was to make seeds more friable to facilitate faster dehulling or removal of seed coat from the surface of cotyledons. The seeds were dehulled manually.

after soaking the grains in fresh tap water.

Seed coat and cotyledon fractions

Hundred grams of whole raw cowpea seeds of each genotype were soaked individually in 200ml of distilled water for 4 hours at room temperature. The seed coat were then carefully removed manually.

Sample preparation

After dehulling, the seed fractions were dried in an aluminium tray in an air oven at a temperature of 50ºC. The seed coats were dried for a period of one hour whereas the cotyledons were dried for two hours. After drying, the cotyledons and seed coats were collected and were ground in Wiley mill. Ground samples of seed coat and cotyledons were passed through the 60 mesh sieve so as to obtain the uniformity of the sample for different estimations. These milled seeds were then kept in air tight containers at room temperature till further analysis. In all the estimations the readings were taken in triplicate.

Sample analysis

Moisture content

Moisture content of seed coat and cotyledon fractions was estimated by air oven method (AOAC, 1995).

Colour of seed coat

Colour of seed coat of each genotype was determined using Munsell soil colour chart (1954) and it was reported as hue, values and chroma.

Total phenol content

The cowpea seed coats and cotyledons were analyzed for total phenol content using the Folin-ciocalteau method given by Mallick and Singh, (1980). One gram of sample was extracted with 10ml of 80% ethanol in a pestle and mortar at room temperature. The homogenate was centrifuged at 10000rpm for 20 min. The supernatant was preserved. The residues were further extracted by adding the same solvent and the extraction procedure was repeated for five times. The pooled supernatant was evaporated to dryness in a rotary vacuum evaporator. Then, added 5ml of distilled water to the dried supernatant.

An aliquot of 0.2ml was taken in a test tube and 3 ml of distilled water was added followed by addition of 0.5ml of Folin-ciocalteau reagent and after 3 minutes, 2ml sodium carbonate (20%) was also added. The reaction mixture was kept in boiling water bath for 1min. The absorbance was measured at 650nm in a spectrophotometer. Total phenol content was expressed as Gallic acid equivalents.

The total phenol (mg GAE/ 100g) was calculated by using following formula:

$$mg\ GAE/100g = \frac{Standard\ Concentration}{O.D\ of\ Standard} \times \frac{O.D\ of\ Sample}{Aliquot\ Taken} \times \frac{Total\ Volume\ made}{Sample\ Taken\ (g)} \times \frac{100}{1000}$$

Antioxidant activity assay using DPPH radical

Antioxidant activity was determined by scavenging of the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method of Brand-Williams *et al*. (1995). Ten grams of homogenized sample was extracted using 15 ml of 80% methanol: distilled water (v/v) in shaker for 30 minutes at room temperature. The extracts were centrifuged at 4000 rpm for 10 minutes and supernatant were collected in separate flask. The residues were further extracted by adding the same solvent and the extraction procedure was repeated for three times. Volume made to a round number of collected extracts and samples were stored at -20°C and used for antioxidant activity analysis. To 0.2ml of diluted extract and 4ml of 0.1mM DPPH solution (prepared freshly) was added and further incubated for 20 min at 37 °C in a water bath, and then the decrease in absorbance at 517 nm was measured (AE). A blank sample containing 100 µL of methanol in the DPPH solution was prepared daily, and its absorbance was measured (AB). The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

% inhibition = [(AB - AE)/AB] × 100

Statistical analysis

Results were expressed as means ± standard deviation (SD) of three measurements. Statistical analysis was performed using ANOVA and $p<0.05$ was considered to be significant. Correlations among data obtained were calculated using the

MS Excel software correlation coefficient statistical option.

RESULTS AND DISCUSSION

Seed coat characteristics

Table 1 represents the morphological characteristics for cowpea seed coat. Fractions of seed coat varied from 12.22±0.03g (PGCP-13) to 13.91±0.03g (PGCP-11). Ehiwe (1987) reported value of 3.5-11.2 per cent seed coat in 11 different varieties of cowpea whereas Sanni et al. (2006) reported a range of 0.3-1.0 per cent.

Moisture and dry matter content in seed coat of different genotypes of cowpea also estimated (Table 1). The values for moisture content in seed coat of various cowpea varieties ranged from 5.63±0.59 (PGCP-11) to 6.66±0.38 (PL-2) per cent. Sasanam (2011) reported moisture content in cowpea with a range of 7.80±0.17 to 9.94±0.06 per cent moisture content in whole seed while 8.80±0.05 to 9.60±0.04 per cent in seed coat.

Dry matter for seed coat of four cowpea genotypes ranged from 94.45±0.37 (PL-2) to 95.35±0.22 (PGCP-11) per cent. The fractions of seed coat, moisture and dry matter content of seed coat varied significantly ($p<0.05$) with respect to variety.

Cotyledon characteristics

In cotyledons, fraction varied from 86.07±0.03g (PGCP-11) to 87.76±0.02g (PGCP-13) on as is basis (Table 2). All the experimental cowpea genotypes varied significantly ($p<0.05$) from each other with respect to cotyledon content. Ali (2007) concluded that seed coat and cotyledons in pulses were 11 and 87 per cent, respectively.

The values for moisture content of cotyledons of four genotypes of cowpea ranged from 5.53±0.24 (PGCP-13) to 6.36±0.44 (PL-2) per cent (Table 2). Dry matter in cotyledons ranged from 93.25±0.36 (PGCP-13) to 94.75±0.31 (PGCP-12) per cent. Variation in moisture and dry matter of different genotypes may be attributed to genetic variation (Table 2).

Colour of seed coat

The genotype of cowpea were analysed for their colour, value, hue and chroma (Table 3). PGCP-13 was found to have the lightest colour (pale olive) while the darkest colour was recorded for genotype PGCP-11 (black). The hue varied from yellow to red with a range of 2.5Y to 7.5Y in PGCP-11, PGCP-12 and PGCP-13 whereas hue of PL-2 was observed to be 7.5R. The values of different genotypes varied from neutral i.e. N2 in PGCP-11 which was darkest in colour to 6 (PGCP-13) which was lightest among the four varieties. Chroma ranged from 0 (PGCP-11) to 8 (PGCP-12).

Total phenol content

Phenolic compounds are secondary metabolites which are synthesized in plants. They possess biological properties such as: antioxidant, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, and cardiovascular protection. The phenol content in selected varieties of cowpea on dry basis is presented in Table 4. PGCP-11 showed highest amount of total phenol content (455.64±0.51mg GAE/100g) and PGCP-13 had the least amount (254.68±0.39mg/100g) in seed coat (Table 4). Similarly, in cotyledon fraction of cowpea, PGCP-11 (258.81±0.75mgGAE/100g) had the highest amount of total phenol content and PGCP-13 had the least (163.25±0.26mgGAE/100g). All the experimental cowpea genotypes varied significantly ($p<0.01$) for total phenol content in seed coat and cotyledon among them. In the present study the results obtained showed that the seed coat had higher amount of total phenol content in comparison to the cotyledon.

Oboh (2006) reported the phenol content of cowpea in different varieties in the range of 30-120 mg/100 whereas Pasko (2009) reported a range of 295-375 mg/100g in different varieties of pseudocereals viz. quinoa and amaranth. Sreeramulu et al. (2009) reported a range of 62.35 (green gram dhal)-418.34 (black gram dhal) mg/100g phenol content in different pulses. Ogunlade *et al*. (2014) estimated total phenolic content of the cowpea bean varieties and it ranged from 88.19 in *Vigna unguiculata* subsp. sesquipedalis (white colour) to 100.2 mg GAE/100g DW in *Vigna unguiculata* subsp. cylindrica (brown colour) with *Vigna unguiculata* subsp. cylindrica showing significantly higher TPC.

Awika et al. (2011) reported that phenol content varied by a factor of more than 50 among the cowpea samples analyzed (minimum 0.3, maximum 17.0 mg/g GAE). Based on seed coat colour, Awika et al. (2011) reported that the white varieties had the lowest phenol content (mean = 3.1 mg/g, GAE). The black, red, and light brown varieties had the highest phenol content (means = 11.9–14.8 mg/g, GAE). The values obtained in the present study are in accordance with the generally observed trend.

Antioxidant activity

By definition, the antioxidant activity is the capability of a compound (composition) to inhibit oxidative degradation, e.g. lipid peroxidation. The antioxidant activity of seed coat of all the four cowpea genotypes, ranged from 33.94±0.05 (PGCP-13) to 86.31±0.32 (PGCP-11) per cent whereas in cotyledon it ranged from 15.93±0.43 (PGCP-12) to 45.75±0.17 (PGCP-11) per cent (Table 4). In present study, all the four genotypes of cowpea differed significantly from each other with respect to antioxidant activity. In both seed coat and cotyledon, PGCP-11 had the highest amount of antioxidant activity than PL-2, PGCP-12 and PGCP-13.

Oboh (2006) observed that *V. unguiculata* had DPPH free radical scavenging ability in the range of 5.5–29.9% and cowpea variety having white seed coat with the lowest phenol content exhibited the lowest antioxidant activity. Oboh (2006) concluded that one variety of the commonly consumed cowpea *V. unguiculata* (brown) could be considered as a functional food in additional to their traditional role of providing dietary proteins. Sowndhararajan (2010) reported that phenolic compounds and antioxidant activities in legume seeds were most abundant and least expensive sources of protein in human/animal diet. Doss et al. (2011) studied total phenol and antioxidant activity in raw, roasted and germinated seeds in underutilized legumes and reported the value of antioxidant activity in raw seeds to be 0.202-0.336µg/ml whereas 0.263-0.445 µg/ml in roasted and 0.297-0.347 µg/ml in germinated seeds. Sreeramulu et al. (2009) reported that DPPH free radical scavenging ability was highest in rajmah (1.07 mgTE/g) and least in roasted Bengal gram dhal (0.26 mgTE/g).

Relationship between antioxidant capacity and total phenolic content in cowpea seed coat

Correlation between antioxidant activity and phenol content of seed coat and cotyledon of different genotypes of cowpea was calculated and the results are presented in Figure 2. It can be clearly seen from Figure 2, that as the total phenol content increases, antioxidant activity also increases. A highly positive linear correlation was found between total phenol and antioxidant activity in both seed coat and cotyledon. R^2 for total phenol content and antioxidant activity of seed coat was 0.923 at Y=0.221x-17.76 whereas in cotyledon R^2 was 0.869 at Y=0.271x-27.74. The figure 2 reveals that more than 90% (correlation coefficient of 0.923) of the antioxidant capacity of seed coat of the cowpea varieties are contributed by phenolic compounds.

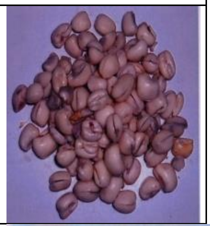
Sl. No.	Geno-types	Seeds	Single grain	Seed coat	Single cotyledon	Cotyledons
1.	PL-2					
2.	PGCP-11					
3.	PGCP-12					
4.	PGCP-13					

Fig. 1: Selected genotypes of cowpea under study

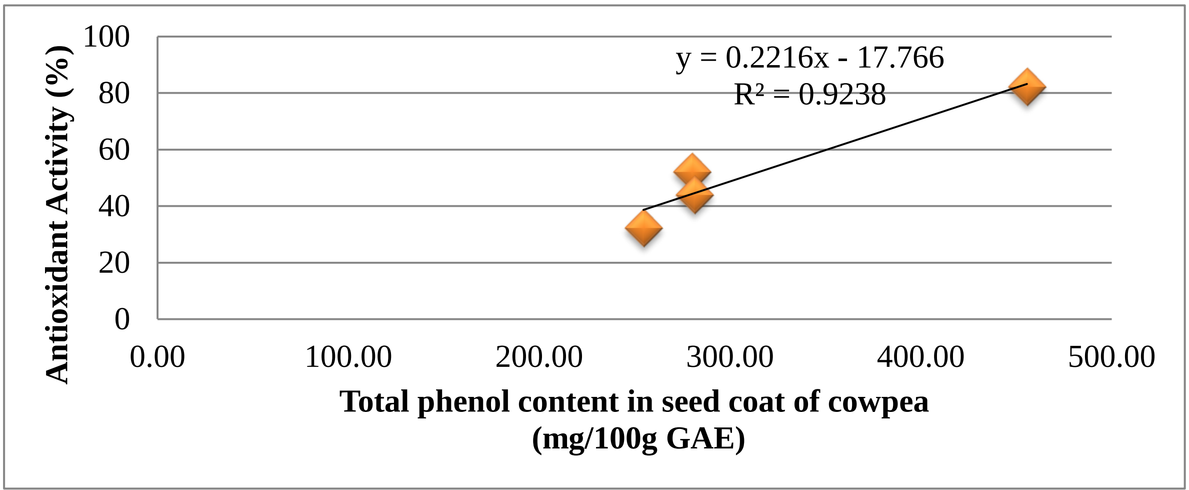


Fig. 2. Relationship between antioxidant capacity and total phenolic content in cowpea seed coat

Table 1: Morphological characteristics of seed coat of selected genotypes of cowpea (per 100g) on as is basis*

Genotype	Seed coat content (g)	Moisture (g)	Dry matter (g)
PL-2	12.32±0.09[b]	**6.66±0.38[a]**	**95.45±0.37[a]**
PGCP-11	**13.91±0.03[a]**	**5.63±0.59[b]**	95.35±0.22[b]
PGCP-12	13.45±0.05[ab]	5.70±0.48[b]	**95.15±0.51[bc]**
PGCP-13	**12.22±0.03[b]**	5.66±0.51[b]	95.30±0.73[b]
C.D. at 5 %	1.22	0.09	0.05

*Mean of triplicate observations ± S.D.; Mean values in a column followed by the same small case letter are not significantly different

Table 2: Morphological characteristics of cotyledons of selected genotypes of cowpea (per 100g) on as is basis

Genotype	Fraction (g)	Moisture (%)	Dry matter (%)
PL-2	87.43±0.08[ab]	**6.36±0.44[a]**	93.92±0.62[bd]
PGCP-11	**86.07±0.03[b]**	5.63±0.27[bd]	94.63±0.73[bc]
PGCP-12	86.54±0.06[ab]	5.73±0.65[bc]	**94.75±0.31[a]**
PGCP-13	**87.76±0.02[a]**	**5.53±0.24[b]**	**93.25±0.36[b]**
C.D. at 5 %	1.45	0.03	0.01

*Mean of triplicate observations ± S.D.; Mean values in a column followed by the same small case letter are not significantly different

Table 3: Hue, value chroma and colour of selected genotypes of cowpea

Genotype	Hue	Value	Chroma	Colour
PL-2	7.5R	3	6	Dark Red
PGCP-11	2.5Y	N2	0	Black
PGCP-12	7.5Y	5	8	Strong Brown
PGCP-13	5Y	6	4	Pale Olive

Table 4: Antioxidant activity and total phenol content on dry basis in selected genotypes of cowpea

Parameters	Total phenol content (mg GAE/100g)		Antioxidant activity (% inhibition)	
Genotypes	**Seed coat**	**Cotyledons**	**Seed coat**	**Cotyledons**
PL-2	281.38±0.40[b]	178.71±0.66[b]	46.58±0.48[c]	24.27±0.38[b]
PGCP-11	**455.64±0.51[a]**	**258.81±0.75[a]**	**86.31±0.32[a]**	**45.75±0.17[a]**
PGCP-12	280.07±0.40[c]	170.19±0.54[c]	54.77±0.39[b]	**15.93±0.43[d]**
PGCP-13	**254.68±0.39[d]**	**163.25±0.26[d]**	**33.94±0.05[d]**	18.12±0.32[c]
C.D. at 5 %	0.12	0.06	0.17	0.04

*Mean of triplicate observations ± S.D.; Mean values in a column followed by the same small case letter are not significantly different

CONCLUSION

The consumption of a cowpea seeds would not only improve nutrient utilization, but also will help in providing potential benefits for human health. Hence, it could be concluded that cowpea could contribute significantly in the management and/or prevention of degenerative diseases associated with free radical scavenging, in addition to their significant role of preventing protein malnutrition.

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