Original Research Paper Food Science

Evaluation of Nutritional, Phytochemical and Antioxidant properties of Cowpea [*Vigna unguiculata* (L.) Walp.]

Shivanki Aggarwal Research Scholar, Department of Food Science and Nutrition, Banasthali University, Rajasthan, India

Sheel Sharma Professor, Department of Food Science and Nutrition, Banasthali University, Rajasthan, India

ABSTRACT Cited as poor man's meat, Cowpea [*Vigna unguiculata* (L.) Walp] is gaining conspicuous recognition as nutraceutical ingredient owing to the presence of an array of phenols and flavonoids, besides being the dietary prowess of traditional nutrients. This study was carried out to evaluate proximate composition, micronutrient content i.e. calcium and iron and antioxidant properties of cowpea. Antioxidant potential of cowpea was endeavoured to be explored in three categories : antioxidant vitamins i.e. vitamin C and β-carotene estimation, phytochemical analysis i.e. total phenols and flavonoids, and antioxidant activity assessment in terms of DPPH (2,2-Diphenyl-1-picrylhydracyl) radical scavenging assay, ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay and FRAP (Ferric reducing antioxidant power) assay. Aqueous-organic (methanol and acetone) extracts of cowpea were prepared for phytochemical and antioxidant analysis. Results reveal cowpea to be an excellent source of protein (23.83%), good source of fibre (3.15%), calcium (67.70mg/100g) and iron (6.76mg/100g). Vitamin C and β-carotene was not found to be in appreciable amounts, which however have a citation to be significantly increased employing household processing methods i.e. germination and fermentation. Cowpea was found to contain phenols (TFC) and flavonoids (TFC) in good amounts, values of TPC and TFC being 135.33 mg GAE/100g and 109.14 mg CE/100g respectively. Also, it demonstrated significant antioxidant activity, values of DPPH, ABTS and FRAP assays being 600.87mg TE/100g, 660.12mg TE/100g and 480.30 mg $FeSO_4$/100g respectively. In snapshot, Cowpea is strongly recommended as a dietary repository of protein, dietary fiber, iron, phenols and flavonoids and as effective chorus to mitigate diseases associated with oxidative stress.

KEYWORDS : .cowpea, phenolics, flavonoids, antiradical efficacy

Abbreviations

LDL: Low Density Lipoprotein, ROS: Reactive Oxygen Species, NF-kB – Nuclear Factor Kappa B, DPPH: 2,2-Diphenyl-1-picrylhydracyl, ABTS: 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), FRAP: Ferric reducing antioxidant power, TPC: Total phenolic content, TFC: Total flavonoid content, TE: Trolox equivalent, GAE: Gallic acid equivalent, CE: Catechin equivalent, TROLOX: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

Introduction

The basic concepts of nutrition are witnessing unprecedented transformation. Recently purported terms like "functional foods", "nutraceuticals", "foods for specific health use" or "specific health promoting foods" suggest that foods may have a beneficial action or certain functions in the organisms that goes beyond the nutritional effects. In this regard, phytochemicals are gaining unprecedented recognition in research community owing to their antioxidant and nutrigenomic properties culminating in health promotion and disease prevention. Phytochemicals are bioactive, non-nutrient, plant-derived chemical compounds and have ubiquitous presence in plants foods i.e. whole cereals and pulses, fruits and vegetables, nuts and oilseeds. Ample evidence indicates that consumption of phytochemicals through natural plant food sources is more effective form health point of view than administration of isolated, high pharmacological doses of antioxidant supplements (Bouayed and Bohn, 2010).

Cited as poor man's meat, Cowpea is gaining the worldwide status of Functional food attributed to being the repository of wide range of phytochemicals besides being rich in conventional nutrients i.e. protein, oilgosaccharides, vitamins, minerals, soluble and insoluble dietary fiber. Nutrient antioxidants in cowpea entail α- and δ-tocopherols, ascorbic acid, β-carotene (Doblado et al, 2005). Phenolics in cowpea belong to flavonoids [proanthocyanidins (eg. monomeric, dimeric, polymeric), Flavan-3-ols (eg. catechin, epicatechin), Flavonols (eg. quercetin, rutin, myricetin, kaempferol glycosides), Flavonones (eg. Naringenin, Naringin), Isoflavones (eg. daidzein, genistein glycosides), Anthocyanins (cyanidin, delphinidin, petunidin, peonidin, malvidin)]; phenolic acids (p-coumaric, ferulic, caffeic, gallic, vanillic, p-hydroxylbenzoic, syringic, cinnamic, sinapic and procatechuic acids) (Adeyemi and Olorunsanya, 2012; Ojwang L, 2012). Various *in vivo* studies have found cowpea to be a promising agent in inhibiting LDL oxidation, inhibiting ROS production, downregulating NF-kB, improving plasma antioxidant capacity and hepatic activity of antioxidant enzymes (Hachibamba et al, 2013, Ojwang L, 2012, Kapravelou et al, 2015).

Materials and Methods

Sample procurement: Variety RC-101 for Cowpea (*Vigna unguiculata* (L.) Walp.; lobhia) was procured from Indian Institute of Pulse Research, Durgapura (Jaipur). It was washed, dried in shade and then hand sorted to remove wrinkled seeds and foreign material and thereafter, stored in air tight container for further use.

Chemicals: Sodium salt of 2,6-Dichlorophenol indophenol dye, sodium bicarbonate, standard vitamin C, 5% metaphosphoric-10% acetic acid solution, ethanol, petroleum ether**,** Folin-ciocalteau reagent, sodium carbonate, gallic acid, aluminium chloride hexahydrate (AlCl3.6H2O), NaNO2, sodium hydroxide (NaOH), catechin, Ethanol, DPPH, ABTS, TROLOX, potassium persulphate, sodium acetate trihydrate, glacial acetic acid, 2,4,6-tripyridyl-S-triazine (TPTZ), Conc. hydrochloric acid (HCl), ferric chloride hexahydrate (FeCl3.6H2O), Ferrous sulphate heptahydrate (FeSO4.7H2O), deionized water were obtained from Sigma Chemicals and Merck.

Sample preparation: the sample was pulverized using home grinder and was used immediately for phenolic extraction.

Nutrient analysis: Moisture, ash and crude fibre of cowpea was determined by AOAC (2000) method. Crude protein and crude fat were determined by the Micro-Kjeldahl and soxhlet extraction method respectively. Total crude carbohydrates were obtained by difference method. Calcium and iron were estimated by following the AOAC (2000) meyhod.

Quantification of nutrient antioxidants:

1) Vitamin C

Vitamin C estimation was done according to method given by Raghuramulu et al 2003

Principle*:* Vitamin C (L-ascorbic acid), after extraction in a mixture of metaphosphoric acid and dilute acetic acid, is estimated by titrating it with 2,6 Dichlorophenol indophenol dye solution. Oxidized form of this dye has a blue and red colour in alkaline and acidic medium respectively while reduced form has no colour (leuco form). Upon redox reaction with vitamin C, the dye generates its leuco form.

Sample extraction- 10g sample was homogenized with about 50ml of 5% metaphosphoric-10% acetic acid solution. Then it was transferred into a 100ml volumetric flask and was shaken gently until a homogeneous dispersion was obtained. Then it was diluted up to the mark by 5% metaphosphoric-10% acetic acid solution. Then the solution was filtered and the clear filtrate was collected for the determination of vitamin C in that sample

Sample analysis- The burette was filled with dye solution (52mg of sodium salt of the dye + 42mg of sodium bicarbonate in water and made up to 500ml). 20ml of standard vitamin C solution (10mg/L in 5% metaphosphoric-10% acetic acid solution) was transferred into a titration flask and titrated against the dye solution to the appearance of a light pink color. Noted down the volume. Similarly, 20ml of test solution was titrated against the dye solution and value was noted.

Calculations*:* Since the concentration of 20ml standard vitamin C solution is 200µg, vitamin C content in 100ml or 10g test sample is calculated as $\frac{Y}{X} \times 1mg$, where Y= amount (ml) of dye causing oxidation of test vitamin C solution (20ml) and X= amount (ml) of dye causing oxidation of standard vitamin C solution (20ml).

1) β-carotene

β-carotene estimation was done in accordance with Mustafa and Babura, 2009

Procedure*:* 10g of macerated sample was placed into a conical flask containing 50ml of 95% ethanol and maintained at a temperature of 70-80°C in a water bath for 20 minutes with periodic shaking. The supernatant was decanted, allowed to cool and its volume was measured by means of a measuring cylinder and recorded as initial volume. The ethanol concentration of the mixture was brought to 85% by adding 15ml of distilled water and it was further cooled in a container of ice water for about 5 minutes. The mixture was transferred into a separating funnel and 25ml of petroleum ether was added and the cooled ethanol was poured over it. The funnel was swirled gently to obtain a homogeneous mixture and it was later allowed to stand until two separate layers were obtained. The bottom layer was run off into a beaker while the top layer was collected into a 250ml conical flask. The bottom layer was transferred into the funnel and re-extracted with 10ml petroleum ether for 5-6 times until the extract became fairly yellow. The entire petroleum ether was collected into 250ml conical flask and transferred into separating funnel for re-extraction with 50ml of 80% ethanol. The final extract was measured, its absorbance was measured using a spectrophotometer at a wavelength of 436nm.

Calculations*:* Equation of Bear-Lamberts law was applied i.e. A=ECL or C=A/EL; where C= concentration of carotene, A= absorbance, E= extinction coefficient, L= thickness of cuvettes (1 cm), E = $1.25x10^4$µg/l

Sample extraction for phytochemical and antioxidant analysis*:*

Pulverized samples (0.25g) were placed in test tubes with 10ml of methanol/water (50:50, v/v). The pH was adjusted to 2 using 2M HCl. The tubes were thoroughly shaken, using orbital shaker, at room temperature for 1 hour, and then centrigued at 2500 g for 10 minutes. Supernatants were collected in clean dry test tubes. Then the residues were extracted again with 10 ml of an acetone/water mixture (70:30, v/v). The methanol and acetone extracts were combined and subsequently used for various assays. Extracts produced in duplicate. In case of non-usage of extracts on the same day, they were stored at 4°C and used within a week for all analysis.

Phytochemical analysis*:*

1) Determination of Total phenolic content (TPC)

Principle: It is based on the single electron transfer (SET) in alkaline medium (7% $NaCO_3$) from phenolic compound to molybdenum, forming blue complex which is measured spectrophotometrically at 750-765nm.

Procedure: TPC was determined according to Xu and Chang, 2007 using Folin-Ciocalteu assay and having gallic acid as standard. The mixture of sample solution (0.1 ml), deionized water (6 ml), FCR solution (0.5 ml), 7% $NaCO_3$ (1.5 ml) was vortexed for 1 min and incubated for 8 min at room temperature. Then a dose of 1.9 ml of deionized water was added. The mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm against blank.

Calculations: TPC was expressed as gallic acid equivalent (mg GAE/100g sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 100 to 1000 µg/ml.

2) Determination of Total flavonoid content (TFC)

Principle: Aluminum chloride forms acid complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonois. In addition, aluminum chloride forms acid labile complexes with orthodihydroxyl groups in the A or B ring of flavonoids.

Procedure: TFC was determined according to Xu and Chang, 2007. 0.5 ml of sample was mixed with 2.5 ml of deionized water in a test tube followed by adding 0.15 ml of 5% sodium nitrite ($NaNO_2$) solution. After 6 minutes, 0.3 ml of 10% aluminum chloride hexahydrate ($AlCl_3.6H_2O$) solution was added and allowed to stand for another 5 minutes before adding 0.1 ml of 1 M NaOH. The mixture was brought to 5 ml with the addition of 0.55 ml of deionized water and mixed well. The absorbance was measured immediately against the blank (the same mixture without the sample) at 510 nm using UV-spectrophotometer.

Calculations: TFC was expressed as catechin equivalents (mg CE/100g sample). Linearity range of catechin calibration curve was 62.5-375 µg/ml.

Antioxidant activity analysis*:*

1) DPPH radical scavenging activity

Principle: It is based on antioxidant-catalyzed reduction of purple-colored DPPH radical to its yellow-colored non-radical form which is measured spectrophotometrically at 517nm.

Procedure: This assay was conducted according to Xu and Chang, 2007. A dose of 0.2 ml of tested legume extract was added to 7.6 ml ethanol solution of DPPH radical (final concentration was 0.1 mM). The mixture was shaken vigorously for 1 min by vortexing and left to stand at room temperature in the dark for 30 min. Thereafter, the absorbance was measured at 517 nm against ethanol blank.

Calculations: DPPH scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linearity range of trolox calibration curve was **0**.05 µM to 0.4 mM trolox.

2) ABTS radical scavenging activity

Principle: It is based on antioxidant inhibition of the absorbance of blue-green coloured ABTS radical, generated via persulfate-induced ABTS oxidation, measured spectrophotometrically at 734nm.

Procedure: This assay was conducted according to Thaipong et al., 2006. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and

allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^{+}$ solution with 30 ml methanol to obtain an absorbance of 1.1 ±0.02 units at 734 nm using the spectrophotometer. In sample (0.3 ml), prepared $ABTS^{+}$ solution (5.7 ml) was added and kept it for 2 hrs in a dark condition. Then the absorbance was taken at 734 nm using spectrophotometer.

Calculations: ABTS scavenging activity was expressed as trolox equivalents (mg TE/100g sample). Linaerity range of trolox calibration curve was 0.1-0.7 mM trolox.

3) FRAP assay

Principle: It is based on antioxidant-catalyzed reduction of ferric-TPTZ complex (colorless) to ferrous form (intensely blue coloured) which is measured spectrophotometrically at 593nm

Procedure: This assay was conducted according to Xu and Chang, 2007. The working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10mM TPTZ (2,4,6-Tripyridyl-s-triazine) in 40mM HCl and with 1 volume of 20mM FeCl3 ×6H2O (ferric chloride hexahydrate). Prepared working FRAP reagent was warmed to 37 C. In 6 ml FRAP reagent, 0.2 ml of sample and 0.6 ml of deionized water were added and the absorbance was taken at 593 nm against reagent blank after 4 min.

Calculations: FRAP value was expressed as Fe^{2+} (Iron (II) sulfate heptahydrate or ferrous sulfate) equivalent (mg FRAP/100g sample). Linearity range of the calibration curve was 0.1-0.9 mM.

Results and Discussion

The quantum of nutrition supplied by cowpea [*Vigna unguiculata* (L.) Walp.] is astounding. Known to be excellent in protein, it is a prosperous food source of carbohydrates of which soluble dietary fibre, insoluble dietary fibre and oligosaccharides form a significant proportion. It is low in fat content too. In present study the protein content of cowpea was found to be 23.83%, carbohydrate value was 57.24%; while fat, fibre and ash content have been surfaced as 1.4%. 3.15% and 3.53% respectively, as shown in Table 1. Pertaining to micronutrients, cowpea is cited to be rich in vitamins and minerals i.e. folic acid, calcium, magnesium, potassium, phosphorus, iron, sodium. This study revealed calcium and iron values of cowpea as 67.70 mg/100g and 6.76±0.17 respectively, as shown in table 2. This result is noteworthy since over 60% world population is iron deficient (White and Broadley, 2009). The results are in line with those demonstrated by Gopalan et al, 2002.

Table 1: Proximate composition of Cowpea

Moisture (g%)	Protein (g%)	Fat (g%)	Fibre (g%)	Ash (g%)	Carbohydrate (g%)
10.82±0.05	23.83±0.55	1.4±0.14	3.15±0.51	3.53±0.18	57.24±0.44

Values are mean±standard deviation, n=3

Table 2: Micronutrient content of Cowpea

Calcium (mg/100g)	Iron (mg/100g)
67.70±0.58	6.76±0.17

Values are mean±standard deviation, n=3

Exploration of antioxidant vitamins in cowpea reveal this legume to be a dietary source of α-tocopherol, δ-tocopherols, ascorbic acid and β-carotene (Doblado et al, 2005). The vitamin C and β-carotene results of cowpea shown in table 3 depict them not to be abundant in these antioxidant vitamins, mean values being 3.04mg/100g and 10.33µg/100g. The results are in agreement with Gopalan et al, 2002. However, numerous evidence has reported germination and fermentation of cowpea to bring exponentially significant improvement in ascorbic acid, β-carotene content and associated antioxidant activities (Doblado et al, 2007). An *In vivo* (mice) study by Kapravelou et al, 2015 pin-points fermented cowpea to have led to improved plasma antioxidant activity and hepatic activity of antioxidant enzymes. Therefore, cowpea can potentially be used as good dietary sources of antioxidant vitamins by preparing germinated and fermented recipes from its raw seed.

Table 3: Antioxidant vitamins content of Cowpea

Vitamin C (mg/100g)	B-carotene (µg/100g)
3.04±0.08	10.33±0.57

Values are mean±standard deviation, n=3

Phytochemical analysis of cowpea demonstrated it to be a good source of phenols and flavonoids as shown in table 4, values being 135.33mg gallic acid equivalent/100g and 109.14 mg catechin equivalent/100g. A study by Yadav et al, 2015 on antioxidant properties of methanolic extracts prepared from four cowpea cultivars exhibited the consistent TPC and TFC results, expressed in the range of 78-187 mg GAE/100g and 84-116mg quercetin equivalent/100g respectively. Moreover, Germination of cowpea has been found to witness significant increase in phytochemicals i.e. phenolics, condensed tannins, catechins and associated antioxidant activity which hypothetically attributes to the activation of polyphenol oxidase during germination leading to mobilization of bound phenolic compounds and generation of new flavonoids (Aguilera et al, 2013).

Table 4: Phytochemical content of aqueous-organic extracts of Cowpea

TPC (mg GAE/100g)	TFC (mg catechin/100g)
135.33±21.93	109.14±9.22

Values are mean±standard deviation, n=3

The antioxidant activities of cowpea extracts as evaluated by DPPH, ABTS and FRAP methods revealed significant antioxidant potential of cowpea. The results of antioxidant activity (DPPH, ABTS, FRAP) are shown in table 5 which are in agreement with Zia-Ul-Haq et al, 2013; Doblado et al, 2007; and Petchiammal and Hopper, 2014 respectively. This suggests that the extracts of cowpea have radical scavenging and reducing ability required to save us from free radical damage and associated diseases i.e. diabetes, cancer, cardiac problems in which oxidative stress has been deciphered to be a major culprit. The antioxidant activity of this legume is mainly due to wide range of phenolics and flavonoids present in significant amounts.

Table 5: Antioxidant activity of aqueous-organic extracts of Cowpea

DPPH (mg TE/100g)	ABTS (mg TE/100g)	FRAP (mg FeSO4/100g)
600.87±11.97	660.12±8.78	480.30±17.50

Values are mean±standard deviation, n=3

Conclusion

Cowpea [*Vigna unguiculata* (L.) Walp is a rich source of conventional nutrients, particularly protein, beneficial carbohydrates i.e. oligosaccharides, dietary fibre and iron. Recent literature decipher this legume to be much more beyond its conventional richness. Besides being a source of antioxidant vitamins i.e. vitamin C, tocopherols, β-carotene, Cowpea is extolled to contain wide range of phenolics and flavonoids known to provide antioxidant protection in human body via various mechanisms i.e. free radical scavenging, antioxidative cellular signaling pathways, reduction pathways etc. This study endeavoured to evaluate nutrient, phytochemical and antioxidant properties of cowpea validates that this legume proves its mettle in terms of nutrient richness, phytochemical and antioxidant efficacy, making it a potent nutraceutical substance, with potential ability to mitigate the risk of chronic and degenerative diseases.

References

1. Adeyemi, K.D., and Olorunsanya, A.O. (2012), Comparative Analysis of Phenolic

Composition and Antioxidant Effect of Seed Coat Extracts of Four Cowpea (Vigna unguiculata) Varieties on Broiler Meat, Iranian Journal of Applied Animal Science, 2(4), 343-349.
2. Aguilera, Y., Díaz, M.F., Jimenez, T., Benítez, V., Herrera, T., Cuadrado, C., Martín-Pedrosa, M., and Martín-Cabrejas, M.A. (2013). Changes in Nonnutritional Factors and Antioxidant Activity during Germination of Nonconventional Legumes, Journal of Agricultural and Food Chemistry, 61, 8120−8125.
3. AOAC (2000). Official Methods of Analysis, (17th edn.), Official Methods of Analysis of Association of Official Agricultural Chemists, International, Gaithersburg, MD.
4. Bouayed, J., and Bohn, T. (2010), Exogenous antioxidants – Double-edged swords in cellular redox state, Oxidative Medicine and Cellular Longevity, 3(4), 228-237.
5. Doblado, R., Frias, J., and Vidal-Valverde, C. (2007), Changes in vitamin C content and antioxidant capacity of raw and germinated cowpea (Vigna sinensis var. carilla) seeds induced by high pressure treatment, Food Chemistry, 101, 918-923.
6. Doblado, R., Zielinski, H., Piskula, M., Kozlowska, H., Muñoz, R., Frías, J. and Vidal-Valverde, C. (2005), Effect of processing on the antioxidant vitamins and antioxidant capacity of Vigna sinensis Var. Carilla, Journal of Agricultural and Food Chemistry, 53(4), 1215-22.
7. Gopalan, C., Rama Shastri, B.V. and Balasubramanian, S.C. Revised and updated by Narasinga Rao, B.S., Deosthale, Y.G. and Pant, K.C. Nutritive Value of Indian Foods, (2002 Edition), National Institute of Nutrition, Hyderabad, India
8. Hachibamba, T., Dykes, L., Awika, J., Minnaar, A. and Duodu, K.G. (2013), Effect of simulated gastrointestinal digestion on phenolic composition and antioxidant capacity of cooked cowpea (Vigna unguiculata) varieties, International Journal of Food Science and Technology, 48, 2638–2649.
9. Kapravelou, G., Martínez, R., Andrade, A.M., López ,C.C., López-Jurado, M., Aranda, P., Arrebola, F., Cañizares, F.J., Galisteo, M. and Porres, J.M. (2015), Improvement of the antioxidant and hypolipidaemic effects of cowpea flours (Vigna unguiculata) by fermentation: results of in vitro and in vivo experiments, Journal of the Science of Food and Agriculture, 95(6), 1207-16.
10. Mustafa, Y. and Babura, S.R. (2009), Determination of carbohydrate and β-carotene content of some vegetables consumed in Kano Metropolis, Nigeria, Bayero Journal of Pure and Applied Sciences, 2(1), 119-121.
11. Ojwang, L. (2012), Antiinflammatory properties of cowpea phenotypes with different phenolic profiles, Doctorate of Philosophy, Texas A and M university.
12. Petchiammal, C. and Hopper, W. (2014), Antioxidant activity of proteins from fifteen varieties of legume seeds commonly consumed in India, International Journal of Pharmacy and Pharmaceutical Sciences, 6(2), 476-479.
13. Raghuramulu, N., Nair, K.M., and Kalyanasundaram, S. (2003), A Manual of Laboratory Techniques, NIN, Indian Council of Medical Research, Hyderabad, 176-184.
14. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. and Byrne, D.H. (2006), Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts, Journal of Food composition and analysis, 19, 669-675.
15. White, P.J. and Broadley, M.R. (2009), Biofortofication of crops with seven minera elements often lacking in human diets-iron, zinc, copper, calium, magnesium, selenium and iodine, New Physiologist, 182(1), 49-84.
16. Xu, B.J. and Chang, S.K.C. (2007), A comparative study on phenolic profiles and antioxidant activity of legumes as affected by Extraction solvents, Journal of Food Science, 72(2), S159-S166.
17. Yadav, N., Kaur, D., Malviya, R. and Rathore, B.S. (2015). Evaluation of the nutritional, antinutritional and antioxidant properties of selected cowpea (Vigna Unguiculata) cultivars, International Journal of Food and Nutritional Sciences, 4(4), 124-130.
18. Zia-Ul-Haq, M., Ahmad, S., Amarowicz, R. and Feo, V.D. (2013). Antioxidant activity of the extracts of some cowpea (Vigna unguiculata (L) walp.) cultivars commonly consumed in Pakistan, Molecules, 18, 2005-2017.