Research Article

Screening of Pigeonpea *(Cajanus cajan* L.*)* Seeds for Study of their Flavonoids, Total Phenolic Content and Antioxidant Properties

Savita Rani*[1], Gagan Poswal[1], Rajesh Yadav[2], M.K.Deen[1]
[1]Department of Chemistry & Biochemistry, CCS Haryana Agricultural University, Hisar, India
[2]Pulses Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, India.
***Corresponding author's E-mail:** savita0129@gmail.com

Accepted on: 06-08-2014; **Finalized on:** 30-09-2014.

ABSTRACT

Legumes are an excellent source of compounds having antioxidant, antimutagenic and antiaging activities. Antioxidant based drugs/formulations for prevention and treatment of complex diseases like cancer have attracted a great deal of research interest in natural antioxidants. Therefore, in this study, methanolic crude extract of seed coat, dehulled dal, cooked dal, whole seed were analysed for total phenolics, flavonoids and antioxidant properties of pigeopea (*Cajanus cajan* L.). It is a health beneficial pulse crop grown in India. Total phenolic contents were determined using a spectrophotometric technique, based on Folin-Ciocalteau reagent and calculated as Gallic Acid equivalents per gram of extract. Hydrophillic and hydrophobic phenols were also estimated. Aluminium chloride colorimetric method was used for flavonoids determination. *In-vitro* antioxidant activities were screened by ferric thiocyanate (FTC) assay. Further, the free radical-scavenging activities of the extracts were measured as decolourizing activity by trapping of unpaired electron of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The scavenging efficiencies of the extracts increased with the increasing concentrations. The extract of seed coat showed maximum antioxidant activity with EC_{50} value 0.26±0.01 mg/ml of extract by DPPH method and 0.59±0.04 mg/ml of extract by FTC method. This maximum antioxidant activity was positively correlated with maximum total phenolic contents observed in seed coat. Results showed that dehulling and cooking reduced the phenolic contents, consequently antioxidant properties too. Based on the observations, it can be concluded that pigeonpea seeds have a potential source of antioxidants of natural origin & applicable in neutraceutical industries.

Keywords: Pigeonpea, antioxidant, flavonoids, phenolics, free radical scavenging activity.

INTRODUCTION

Availability of proteins in developing countries at present is an indispensable requisite because of ever growing population. Legumes are nutritious food and a substitute for animal protein. Among the legumes, pigeonpea or red gram (*Cajanus cajan* L.) occupies an important place in tropics and subtropics. Today, in terms of global production of legume crops, pigeonpea is sixth after *Phaseolus* species, peas, chickpeas, broad beans and lentils. It is cultivated in 22 countries. India is one of the major producer.[1] In India, dehulled split cotyledons of pigeonpea seeds are cooked to make dal. Its broken seeds, skin & pod walls are fed to domestic animals. Pigeonpea seeds are made up of 85% cotyledons, 14% seed coat, 1% embryo and contain a variety of dietary nutrients.[2] Pigeonpea is also used as integral part of traditional folk medicine. Fresh seeds help incontinence of urine in males while immature seeds are recommended for treatment of kidney diseases.[3] In Africa, pigeonpea seeds are used for treating hepatitis & measles while in China, these are used to arrest bleeding, relieve pain, kill worms and as an expectorant.[4] Seed extract of pigeonpea helps in inhibiting the sickling of red blood cells and therefore has potential to treat the person suffering from sickle cell anaemia.[1] Research efforts revealed that some polyphenols, especially flavonoids, plays an important role in treatment of such diseases because of their high antioxidant action.[5,6]

Phenolics are secondary metabolites widely distributed in plant kingdom. In case of legumes, the distribution of phenolic compounds differs in cotyledons and seed coat.[7] Studies have shown that phenols comprising phenolic acids, flavonoids, anthocyanins possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic as well as the ability to modify the gene expression.[8] Antioxidants are compounds that can delay or inhibit the oxidation of lipids by inhibiting the initiation or propagation of oxidative chain reactions.[9] The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing & neutralizing free radical, quenching singlet & triplet oxygen, decomposing hydrogen peroxides.[10] These free radical reactions cause human diseases and deterioration of food in food industries. Hence, the biomedical scientists and nutritionists are interested in natural antioxidants which can protect body against damage by reactive oxygen species. Many leguminous plants have shown potent antioxidant activity. The literature survey showed scanty information available regarding antioxidant activity of pigeonpea seeds. Therefore, the objectives of this study were to determine antioxidant activity of methanolic extracts of seed coat, dehulled dal, cooked dal and whole seed of pigeonpea to explore relationship between antioxidant activity and antioxidant content in the sample. This study would offer basic data on natural antioxidant potential of pigeonpea seeds for

food and a scientific reference for exploitation of pigeonpea as a resource for large scale usage.

MATERIALS AND METHODS

Plant material

A bulk of healthy seeds of pigeonpea (var. Manak) was procured from Pulses section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar (India). These seeds were sorted manually, to remove stones, damaged and immature seeds. After sorting, major portion of these were dehusked to obtain seed coat and dal. Dehusked dal was divided into two parts. The first part was kept for extraction and second part was cooked in distilled water in ratio of 1:10 (w/v) at 100C, dried at 55°C. Thus, we obtained four samples of pigeonpea, 100 gm each for further analysis.

Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merk and Ranbaxy, of high purity were used for various experimental procedures.

Preparation of extracts

All of the four samples were ground in an electric grinder and converted into fine powder form. The powdered samples were then extracted separately with methanol by refluxing six hours. Extracts were filtered and filterates were concentrated under reduced pressure on rotatory evaporator at 40°C. These concentrated filterates were stored at 5°C for further analysis.

Determination of total phenolic content

The total phenolics were determined by the Folin-Ciocalteu reagent using gallic acid as standard.[11] Appropriately diluted extracts (1ml) were added to a 50 ml volumetric flask filled with 1.0 ml of Folin–ciocalteau reagent (diluted to 1:2 ratio) and 2.0 ml of Na_2CO_3 (20% w/v) were mixed and the volume was made to 50 ml. The mixture was allowed to stand for 50 min. and centrifuged at 6000 rpm for 10 minutes. The absorbance of supernatant solution was measured at 730 nm using Shimadzu UV-Vis spectrophotometer (UV-2600) and against a blank prepared similarly with the same solvent but omitting the extract. The concentration of phenolics thus obtained was multiplied by the dilution factor and the results were expressed as equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

Determination of content of hydrophilic and hydrophobic phenolics

The 50 ml of crude extracts of each of four samples were fractionated into its hydrophilic and hydrophobic components by mixing with 100 ml of deionised water and 100 ml of n-butanol in separating funnel as per Wettasinghe's method.[12] The mixture was allowed to stand till layers got separated. The separated layers were concentrated using rotavapor set at 40°C. Weight of each fraction was recorded and the content of phenolics in each fraction was determined as per Shahidi and Naczk method.[11]

Determination of flavonoids

The aluminium chloride colorimetric assay, as described by Zhishen *et al.* was used.[13] Briefly, 1 ml of diluted extracts were added to test tubes containing 4 ml of double distilled water. A blank solution using doubled distilled water instead of sample was prepared. After five minutes, 0.3 ml 5% $NaNO_2$ was added to the test tubes, followed by 0.3 ml 10% $AlCl_3$. Immediately, 2 ml 1M NaOH was added and the total volume was made upto 10ml with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm vs the prepared blank. Total flavonoids of samples were expressed as mg catechin equivalent per gram of the extract (mg CAE/g).

2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activity of the extracts was determined by DPPH free radical scavenging method. The antioxidant effect of extracts on DPPH free radical was estimated as per method of Hatano *et al*[11]. Solutions of different concentrations 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg of each extract, were added to methanolic solution of DPPH (0.025 g L^{-1}, 2 ml). The mixture was shaken and the absorbance of the resulting solution was measured by using Shimadzu UV- Vis spectrophotometer (UV-2600) at 1,5,10 min. interval at 515 nm until the reaction reached at its plateau gradually. For each sample separate determinations were carried out. Similarly, a control sample was also prepared. The antioxidant activity was expressed as the percentage of decline of the absorbance after 70 min, relative to the control, corresponding to the percentage of DPPH scavenged. The percentage of DPPH, which was scavenged (% $DPPH_{sc}$) was calculated using:

$$\% DPPH_{sc} = \{(A_{cont} - A_{samp})/A_{cont}\} \times 100$$

Where A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

Ferric thiocyanate (FTC) method

The FTC method of Kikuzaki and Nakatani was used to evaluate the antioxidant activity of the extract.[15] Reagents prepared for this method were 2.51% (w/v) linoleic acid in ethanol, 30% (w/v) ammonium thiocyanate, 0.02 mol/L ferrous chloride in 3.5% (v/v) hydrochloric acid, 75% ethanol and 0.2 M phosphate buffer, pH 7.0 (39.0 ml of 0.2 M solution of monobasic sodium phosphate and 61.0 ml 0.2 M solution of dibasic sodium phosphate were mixed, diluted to a total of 200 ml). A linoleic acid emulsion was prepared by mixing linoleic acid (0.28 g), Tween 20 (0.28 g) and phosphate buffer (50 ml, 0.2 M, pH 7.0). Different test samples of conc. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg of each extract, were mixed with 5 ml of linoleic acid emulsion and final volume made to 10 ml with phosphate buffer (0.2 M, pH 7.0) and incubated at 37°C for 96 hours

(4 days). The mixture prepared as above without the test sample served as control. Aliquots (0.1 ml) were drawn from the incubation mixture at intervals of 24 hour and mixed with 0.1 ml of 30% ammonium thiocyanate, 0.1 ml of 20 mM ferrous chloride in 3.5% HCl and final volume made to 10 ml with 75% ethanol and allowed to stand at room temperature for 3 minutes. The colour developed was measured at 500 nm in a spectrophotometer. This method depends on peroxide formation in the aqueous emulsion of linoleic acid. In this method, the higher the absorbance increase is, the higher the concentration of peroxide formed and hence, the lower the antioxidant activity of the sample tested.

Antioxidant activity was expressed as:

Antioxidant activity (%) = (1- increase in abs. of sample/ increase in abs. of control) x 100

Statistical analysis

Four replicates of each sample were used for statistical analysis and resulting values are expressed as mean± S.D. One way analysis of variance (ANOVA) and F-test were carried out to asses for any significant differences between the means (p<0.05). Correlation analyses of antioxidant activity, flavonoids and total phenolic content were carried out using Pearson correlation programme in Online Statistical Analysis (OPSTAT www.hau.ernet.in).

RESULTS AND DISCUSSION

Content of total phenols, hydrophilic and hydrophobic phenols

Phenolics are aromatic secondary plant metabolites are high-level antioxidants because of their ability to scavenge free radicals. The total phenols, hydrophilic and hydrophobic phenols, flavonoids, radical scavenging activity by DPPH method and antioxidant activity by FTC method, of all methanolic extracts are presented in Table 1. Total phenolic content reported in seed coat crude extract was 23.25±4.8 mg GAE/g extract and it consisted of approx. 40% (w/w) and 60% (w/w) of hydrophilic and hydrophobic phenols (ratio of 2:3,w/w) respectively. Dehusked dal contained 8.07±0.05mg GAE/g extract of total phenol and its hydrophilic and hydrophobic phenols were present in 0.45:1 (w/w) ratio. Thus, seed coat contained much more total phenolic content as compared to dehusked dal.

Further, it varied from cooked dal extract which contained 7.6 ±0.5mg GAE/g extract with hydrophilic to hydrophobic phenol ratio of 0.37:1 (w/w). Whole seed methanolic extract had a little higher content of total phenol with the value of 9.44±0.02mg GAE/g extract and it exhibited a ratio of 3:5 (w/w) for hydrophilic and hydrophobic phenol respectively. Thus, highest amount of phenolic compounds in pigeonpea seed coat extract made it superior for exhibiting greatest antioxidant activity.

Flavonoid contents

Flavones and flavonols are the subgroups of flavonoids. Flavonols are known to act as antioxidant, both as radical scavengers and as metal chelators.[16,17] Here, Table 1 also shows flavonoid contents that were measured by aluminium chloride colorimetric assay. Plant extract with a high level of phenolic content also contains a higher flavonoid content.[18] The methanolic extract of seed coat showed maximum flavonoid content (20.9± 0.8 mg CAE/g extract) followed by whole seed extract (8.65± 0.6 mg CAE/g), dehusked dal extract (4.65± 0.4 mg CAE/g) and minimum in cooked dal extract (4.4± 0.3 mg CAE/g).

DPPH radical scavenging efficiency

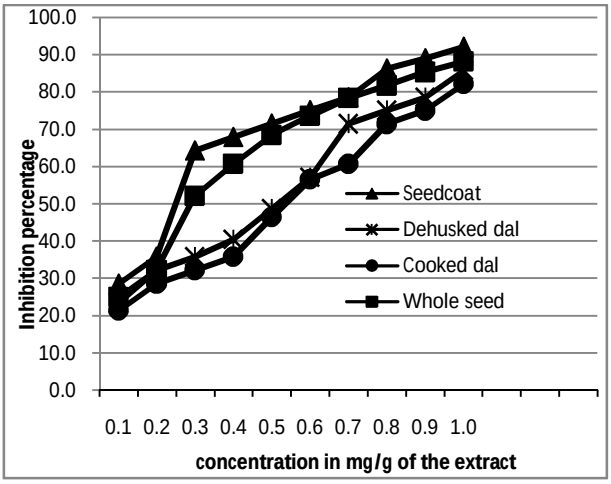
2, 2'-diphenyl-1-picrylhydrazyl radical is one of the few stable and commercially available organic nitrogen radical (DPPH$^{\bullet}$), often used in the evaluation of radical scavenging activity of antioxidants-natural and synthetic pure compounds.[19,20] Alcoholic solutions of DPPH$^{\bullet}$ have a characteristic absorption maximum at 517 nm. When an electron or hydrogen atom donating antioxidant (AH) is added to DPPH$^{\bullet}$ a decrease in absorbance at 517 nm takes place due to the formation of the non-radical form DPPH-H, which does not absorb at 517 nm. This reaction has been measured by the decoloration assay where the decrease in absorbance at 517 nm produced by the addition of the antioxidant to the DPPH$^{\bullet}$ in methanol is measured.

Table 1: Phytochemical contents and antioxidant activity of pigeonpea extracts

Parameters	Seed Coat	Dehusked Dal	Cooked Dal	Whole Seed	BHT
Yield (%) of methanolic extracts	1.96±0.21	6.75±0.27	3.34±0.08	4.06±0.12	_
Total phenolics (mgGAE/g)	23.25 ± 4.8	8.07 ±0.05	7.6 ±0.56	9.44 ±0.02	_
Hydrophilic phenols (mgGAE/g)	14.25 ± 3.1	2.56 ±0.02	1.92 ±0.33	3.52 ±0.33	_
Hydrophobic phenols (mgGAE/g)	8.8±2.1	5.56 ±0.05	5.15 ±0.26	5.95 ±0.26	_
Flavonoids (mgCAE/g)	20.9 ± .8	4.65 ±0.42	4.47 ±.38	8.65 ±0.61	_
DPPH radical scavenging assay EC_{50} (mg/g of extract)	0.26 ±0.01	0.52 ±0.01	0.55 ±0.02	0.29 ±0.02	0.41± 0.01
Ferric thiocyanate (FTC) assay EC_{50} (mg/g of extract)	0.59 ±0.04	0.67 ±0.02	0.71 ±0.05	0.60 ±0.02	0.63±0.02

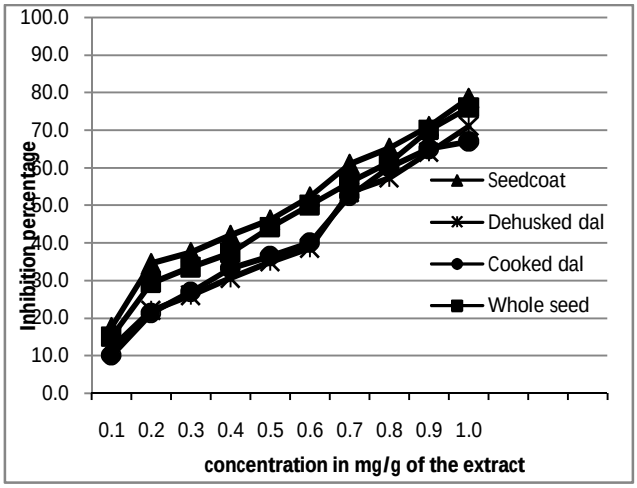
Values are mean of four replicates ± standard deviation and means are different from each other by F-test (p<0.05); mg GAE/g- milligrams gallic acid equivalent/g of the extract; mg CAE/g- milligrams catechin equivalent/g of the extract

All the above described extracts were screened for radical scavenging activity against DPPH•. The antioxidant activity exhibited by methanolic extracts of seed coat, dehusked dal, cooked dal, whole seed and BHT were 91.0±0.01%, 85.7 ± 0.01%, 82.1 ± 0.02%, 87.1±0.02% and 86.0± 0.01 respectively, at the concentration of 1.0 mg/ml of the extract. The corresponding EC_{50} values to scavenge DPPH• radical were 0.26±0.01, 0.52±0.01, 0.55±0.02, 0.29±0.02, 0.41±0.01 mg/g of the extract. The methanolic extract of seed coat showed maximum antioxidant activity in terms of radical scavenging capacity as well as inhibition percentage (Figure 1 & 2).



Values are mean of four replicates ± standard deviation

Figure 1: Inhibition % of all the extracts by DPPH method



Values are mean of four replicates ± standard deviation

Figure 2: Inhibition % of all the extracts by FTC method

Antioxidant activity by Ferric thiocyanate (FTC) Method

The ferric thiocyanate method measures the amount of peroxide produced during initial stages of lipid oxidation, in which peroxide reacts with ferrous chloride and form ferric ions. The ferric ions then combine with ammonium thiocyanate and produce ferric thiocyanate. This substance is red in colour. The darker the colour, higher will be the absorbance.[21] From FTC analysis, it was found that all test samples of crude extracts of pigeonpea had been oxidised when stored for seven days at 37°C. Absorbance of each sample increased by time of incubation. Lower absorbance values at the initial stage showed higher antioxidant activities. With progressing days of incubation, all samples showed higher absorbance and lower antioxidant activity. The % of inhibition or antioxidant activity of methanolic extracts in ascending order is cooked dal (67.0±0.05) < dehusked dal (71.0±0.02) < whole seed (76.1±0.02) < BHT (76.4±1.01) < seedcoat (78.6±0.03). EC_{50} values for all extracts are given in Table 1.

Antioxidant property results from contribution of phenolic compounds which is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donars and singlet oxygen quenchers.[22] There are many secondary metabolites such as volatile oils, flavonoids, anthocyanins etc. which may be responsible for antioxidant properties. The highest radical scavenging activity was shown by seed coat with EC_{50} value 0.26±0.01 mg/g of extract, among all the four samples of pigeonpea. This might be due to hydroxyl groups in the structure of phenolic compound that can provide necessary component as a radical scavenger. Similar relationship between chemical structure of flavonoids and their antioxidant activities had been analysed by Arora *et al.* and Acker *et al.*[23,24] According to them, presence of hydroxyl substituents on flavonoid skeleton boosts activity. But, all the phenolics do not have the same antioxidant activity, some are powerful, others are weak and they develop antagonistic or synergistic effects with themselves or with the other constituents of the extracts.[25-27] In this study, seed coat extract had maximum antioxidant activity alongwith maximum phenolic contents and similar pattern are observed in rest three extracts where the two parameters had corresponding values in same manner. Still it would be difficult to attribute the total antioxidant effect of the extracts to one or a few active principles. Therefore, here we can analyse that antioxidant activity of extracts of pigeopea is probably a synergic effect of their compositions. Following the correlation matrices (p<0.05) of total phenols, flavonoids and their antioxidant activities, which were highly significant, synergistic effect was confirmed.

CONCLUSION

Free radicals are often generated as byproducts of biological reactions or from exogenous factors and these are involved in many human ailments. An efficient scavenger of free radical may serve as a possible preventative intervention for the disease.[28] As per result of this study,whole seed of pigeonpea exhibit high antioxidant property as compared to cooked dehusked dal. Hence, it is advisable to consume dal with seed coat for better antioxidant defense system. We also concluded that extracts of pigeonpea seeds may provide potential natural antioxidant for nutraceutical industry & other fields. Present study could be an effective introduction to the antioxidant properties of pigeonpea and more work

should be done to characterise individual phenolic compounds in order to assign their antioxidant properties.

REFERENCES

1. Saxena KB, Kumar RV, Sultana R, Quality nutrition through pigeonpea—a review, Health, 2, 2010, 1335-1344.
2. Faris DG and Singh U, Pigeonpea: Nutrition and Products, In: Nene YL, Hall SD and Sheila VK, Ed., The Pigeonpea, Wallingford, CAB International, 1990, 401-434.
3. Duke JA, Hand book of legumes of world economic importance, New York: Plenum Press, 1988.
4. Abbiw DK, Useful plants of Ghana, Kew, London, UK: Richmond intermediate technology publications and royal botanic gardens, 1990.
5. Liu HY, Qiu NX, Ding HH and Yao RQ, Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses, Food Research International, 41, 2008, 363-370.
6. Pietta PG, Flavonoids in medicinal plants, In: Rice- Evans CR & Packer L, Ed. Flavonoids in health and disease, New York, Dekker, 1998, 61– 110.
7. Ferreira EC, Nogueira ARA, Souza GB and Batista ARL, Effect of drying method and length of storage on tannin and total phenol concentrations in Pigeon pea seeds, Food Chemistry 86, 2004, 17–23.
8. Tapiero H, Tew KD, Ba GN and Mathe G, Polyphenols: Do they play a role in the prevention of human pathologies? Biomedicine and Pharmacotherapie. 56, 2002, 200–207.
9. Velioglu YS, Mazza G, Gao L & Ooman BD, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products, J of Agri Food Chem, 46, 1998, 4113–4117.
10. Osawa T, Novel natural antioxidants for utilization in food and biological systems, In: Uritani I, Garcia VV and Mendoza EM, Ed. Postharvest biochemistry of plant food-materials in the tropics, Tokyo, Japan: Japan Scientific Societies Press, 1994, 241–251.
11. Shahidi F and Naczk M, Methods of analysis and quantification of phenolic compounds, Food Phenolic: Sources, Chemistry, Effects and Application, Technomic Publishing Company, Inc.: Lancaster, 1995, 287-293.
12. Wettasinghe M, Shahidi F, Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals, Food Chemistry, 70, 2000, 17-26.
13. Zhishen J, Mengcheng T and Jianming W, Determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 64, 1999, 555-559.
14. Hatano T, Kagawa H, Yasuhara T and Okuda T, Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects, Chem.Pharmaceu.Bull., 36, 1988, 2090-2097.
15. Kikuzaki H and Nakatani N, Antioxidant effect of some ginger constituents, J Food Sci, 58, 1993, 1407-1410.
16. Bors W, Saran M, Radical scavenging by flavonoid antioxidants, Free Radic. Res. Commun., 2, 1987, 289-294
17. Afanaslejev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, and Potapovitch AI, Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation, Biochem. Pharmacol., 38, 1989, 1763-1769.
18. Maisuthisakul P, Suttajit M and Pongsawatmanit R, Assessment of phenolic content and free radical scavenging capacity of some Thai indegenious plants, Food Chem., 100, 2007, 1409-1418.
19. Brand-Williams W, Cuvelier ME and Berset C, Use of a free radical method to evaluate antioxidant activity, Lebensmittel-Wissenschatund Technologie 28, 1995, 25–30.
20. Yen GC, Duh PD, Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species, J. Agr. Food Chem, 42, 1994, 629-632.
21. Huda-Faujan N, Noriham A, Norrakiah AS and Babji AS, Antioxidant activity of plants methanolic extracts containing phenolic compounds, African Journal of Biotechnology, 8 (3), 2009, 484-489.
22. Nakatani N, Antioxidants from spices and herbs, In: Shahidi F, Ed. Natural antioxidants: chemistry, health effects, and applications, Champaign, IL, 1997, AOCS Press 64–75.
23. Arora A, Nair MG, Strasburg GM, Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system, Free Radical Biol. Med., 24, 1998, 1355-1363.
24. Acker SABE Van, Van den Berg DJ, Tromp MNJL, Griffioen DH. Van Bennekom WP, Van der Vijgh WJ and Bast A. (1996). Structural aspects of antioxidant activity of flavonoids. Free Radical Biol. Med. 20, 1996, 331-342.
25. Rice-Evans CA, Miller NJ, and Paganga G, Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Radic. Biol. Med., 20, 1996, 933-956.
26. Moran JF, Klucas RV, Grayer RJ, Abian J, and Becana M, Complexes of iron with phenolic compounds from soy-bean nodules and other legume tissues: prooxidant and antioxidant properties, Free Radical Biol.Med., 22(5), 1997, 861-870.
27. Lein EJ, Ren S, Bui HH and Wang R, Quantitative structure-activity relationship analysis of phenolic antioxidants, Free Radical Biol.Med., 26, 1999, 285-294.
28. Doss A, Pugalenthi M, Rajendrakumarand D and Vadivel V, Phenols, flavonoids and antioxidant activity of underutilized Legume seeds, Asian J. Exp. Biol. Sci., 1(3), 2010 700-705.

Source of Support: Nil, **Conflict of Interest:** None.