

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.510.075>

**Evaluation of Total Phenols, Total Flavonoids and *In vitro*
Antioxidant Activity in the Ethanolic Leaf, Seed and Root Extract of
Cajanus cajan (L.) Mill sp**

R. Raveena Devi*, R. Premalatha¹ and R. Kayathri²

Department of Microbiology and Biochemistry, Nadar Saraswathi College of Arts and Science,
Theni - 625 531, Tamil Nadu, India

*Corresponding author email id:

A B S T R A C T

The purpose of the study was to assess total Flavonoids (TF), total phenols (TP) and antioxidant activity (TAA) in the ethanolic leaf, seed and root extract of *Cajanus cajan*. Total phenolic content, Total Flavonoids content were measured by using standard procedures and free radical scavenging activities were performed by using DPPH radical scavenging activity, Hydrogen peroxide radical scavenging and phosphomolybdenum assay. The result indicated that total phenolic content in the analysed leaf, seed and root extracts ranged as 55.03mg\GAE and 57.20mg\GAE, 50.95mg\GAE. Similarly Flavonoids content in the analyzed leaf, seed and root extracts ranged as 36.17 mg\RE\gram, 49.43 mg\RE\gram, and 48.32mg\RE\gram. The outcome of the antioxidant activity were found to be dosage dependent. The IC₅₀ values for DPPH assay for ethanolic extract of leaf, seed and root were 1.407, 0.844 and 1.184mg\ml respectively. The highest hydroxyl radical scavenging activity reached up to 79.07% in seed 76.45% in leaf 75.21% in root extracts respectively. The highest total antioxidant potentials observed in *Cajanus cajan* seed extract compared to other extracts. All the assays tested, it was found that the seed extract possessed highest antioxidant activity. These findings indicated that potential antioxidant activity were found in *Cajanus cajan* leaf, seed and root extracts and needs further exploration for their effective use. The high amount of TFC, TPC, and TAA in the leaf, seed and root of *Cajanus cajan*, makes *Cajanus cajan* effective free radical scavenger and a powerful source of natural antioxidants.

Keywords

Cajanus cajan,
Total Phenols,
Total Flavonoids,
Antioxidant
potential- DPPH,
H₂O₂, Total
antioxidant capacity.

Article Info

Accepted:
20 September 2016
Available Online:
10 October 2016

Introduction

Free radicals such as superoxide radical, peroxide radical and hydroxyl radical take part in a vital role in genesis of diverse degenerative diseases (Long *et al.*, 2007). Antioxidants take part in substantial role in

inhibiting and scavenging free radicals consequently provided that protection to human against degenerative diseases (Ahmad *et al.*, 2006). Antioxidant derived from exogenous source like synthetic antioxidant

compounds, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) which are habitually used in processed foods, it has been reported that these compounds are responsible for liver damage and carcinogenesis (Ghosh *et al.*, 2009).

So, extensive attention has been paid to antioxidant properties of plant products (fruits, vegetables, medicinal herbs, etc). They reducing oxidative stress –induced tissue injury (Pourmorad *et al.*, 2006). Among the abundant naturally occurring antioxidant; ascorbic acid, carotenoids and phenolic compounds are more effectual to scavenge free radicals and active oxygen species.

Cajanus cajan is one of the diploid legume essential crop species belonging to *phaseoleae* family. Tropical and subtropical countries are proper places for growing this plant. The morphological features of this plant are especially erect perennial, annual plant having 1-4m in height. *Cajanus cajan* has been used for therapeutic purpose since time immemorial. Its young pods shoot, leaves, and seeds, roots are edible (Raveenadevi *et al.*, 2016).

Diverse pharmacological activities have been endorsed to extracts of parts of the plant, as well as their phytochemical constituents. It mainly contains Flavonoids, isoflavonoid and stilbenes. Flavonoids has antibacterial, antifungal antiviral and anticancer and anti inflammatory effects. Stilbenes are responsible for the beneficial effects of pigeon pea leaves on human health (Luo *et al.*, 2008).

The extract of pigeon pea leaves are generally used to treat wounds, malaria, bedsores, treat against hypoxic-ischemic brain damage and alcohol-induced liver

damage and also used as antioxidant, antibacterial and anticancer ability. Their seeds have been found to have antisickling activity (Harish *et al.*, 2014). Cajanol isolated from roots of the plant has been used as novel anticancer agent, which induced apoptosis in human breast cancer cells (king *et al.*, 2010).

The antioxidant activity of the extract of the leaves (Wu *et al.*, 2009) and the hypoglycemic activity of the seed have been reported (Nneka *et al.*, 2016).The isolation of antiplasmodial compounds betulinic acid (roots), longistylin A and C (leaves) from the plant reported (Duker-eshun *et al.*, 2004; Zheng *et al.*, 2007).

There is inadequate information about the biological activities of *C.cajan* plant. In view of the folk therapeutic uses of the plant as well as various scientific reports, the goal of the current work was aimed to assess total flavonoids, total phenols and antioxidant activity in the ethanolic leaf, seed and root extract of *Cajanus cajan*

Materials and Methods

Collection of plant parts

The fresh leaves, roots and seeds of *Cajanus cajan L.* were simultaneously collected from cultivated farms of Theni district. Fresh parts of the plants were recognized and authenticated prior to studies. The leaves, Seed & roots were rinsed thrice with distilled water to remove the dust and other contaminants then dried at shade condition for one month. After dry they were grinded into powdered by using a Grinder Machine.

Extraction

Each powdered sample was extracted separately with 100ml of ethanol for 3hrs on

a magnetic stirrer. The yielded solution was filtered through what man No.1 paper. The concentrated extracts were stored in air tight container refrigerated at 4⁰c for the further tests.

Determination of total phenolic content

Total phenolic content of the leaf, seed and root extracts of *C.cajan* was determined using the Folin-cio Calteau reagent. Folin diluted 10 times with distilled water. Ethanolic extract of leaf, seed and root extracts solutions (50µl) was mixed with 1ml diluted Folin-cio Calteau reagent, 1ml sodium bicarbonate solution (7.5%) and 2ml distilled water. The mixture was incubated at room temperature for 15 min. The absorbance of the solution was determined at 730nm using spectrometer and compared with Gallic acid equivalents calibration curve. The total phenolic content was expressed as mg Gallic acid equivalents of *C.cajan*.

Determination of total Flavonoids content

Total flavonoids content of the leaf, seed and root extracts of *C.cajan* was determined according to the aluminium chloride method. In brief, 50µl of leaf, seed and root extract were mixed with 4ml of distilled water and 0.3 ml of 5% NaNO₂ solution, 0.3ml of 10% AlCl₃ solution was added after 5minutes of incubation, and the mixture was allowed to stand for 6minutes. Then 2ml of 1mol\litter NAOH solution were added and the final volume of the mixture was brought to 10ml of double distilled water. The mixture was allowed to stand for 15 minutes and absorbance was measured at 510nm. The total Flavonoids content was calculated from a calibration curve and the result expressed as mg rutin equivalent per gram dry weight.

***In vitro* antioxidant activity evaluation**

DPPH Radicals Scavenging Potentials

DPPH Scavenging activity of ethanolic extract was carried out by Blois 1958.

Cajanus cajan ethanol extract (leaf, seed& root) was mixed with methanol and it was kept under incubation for 24hrs.the sample was centrifuged at 1200rpm for 10minutes and the methanol supernatant was taken for the antioxidant activity studies.

Different concentration of (200-800µg/ml) solvent extracts and ascorbic acid was prepared in methanol .to this 3ml of the extract, 1ml of DPPH solution was added. The reaction mixture was mixed well and incubated at room temperature for 30minutes. After incubation, absorbance was read at 570nm. Ascorbic acid used as a reference compound. The capacity to scavenge the DPPH radical was calculated using the following equations.

The % inhibition was calculated as,

$$\% \text{DPPH scavenging activity} = \frac{(A \text{ control} - A \text{ test})}{(A \text{ control})} \times 100$$

Where a control is the absorbance of the control reaction and A test is the absorbance of the extract. The antioxidant activity of the extract was expressed as IC 50. The IC 50 value is defined as the concentration in µg/ml of extract that inhibits the formation of DPPH radical by %

Total antioxidant capacity test

Antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure described in Prieto *et al.*, 1999.

A 3ml of extract (Leaf, seed & root) was combined with 3ml of reagent solution (0.6M sulphuric acid, 28Mm sodium phosphate and 4Mm ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then absorbance of the solution was measured at 695nm using a spectrophotometer against blank after cooling to room temperature. Ethanol (0.3ml) extract used as the blank. The antioxidant activity is expressed as the number of gram equivalents of ascorbic acid.

Hydroxyl radical scavenging activity

The H₂O₂ radical scavenging activity of ethanolic extract was determined by Ruch *et al.*, 1989.

Procedure

Leaf, seed & root extracts of various concentrations were prepared (200-800µg/ml) in distilled water. 1ml of various concentration of extract was taken in series of test tubes. To each tube 0.6ml of 40mM H₂O₂ solution was added and incubated for 10minutes in room temperature. The absorbance of sample was read at 230nm against a blank solution containing the phosphate buffer without H₂O₂. ascorbic acid was used as a standard antioxidant.

The % inhibition was calculated as,

$$\% \text{ H}_2\text{O}_2 \text{ scavenging activity} = \frac{(\text{A control} - \text{A test})}{(\text{A control} \times 100)}$$

Where a control is the absorbance of the control reaction and A test is the absorbance of the extract. The antioxidant activity of the extract was expressed as IC 50. The IC 50 value is defined as the concentration in µg/ml of extract that inhibits the formation of radical by%.

Statistical Analysis

Mean value of phenols mg/g Dry Weight and Flavonoids mg/g Dry Weight were compared using independent t-test. Comparison of anti-oxidant properties of Ascorbic acid, *Cajanus cajan*, different antioxidant Assay was done using one way analysis of variance (ANOVA). *P-value* less than 0.5 were considered statistically significant.

Result and Discussion

Total phenolic content

Total phenolic content of ethanolic extract of leaf, seed and root of *Cajanus cajan* was evaluated by using the Folin-ciocalteu colorimetric method. The phenolic content in the analysed leaf, seed and root extracts ranged as 55.03mg\GAE and 57.20mg\GAE, 50.95mg\GAE (Table 1). The highest content of phenols was assessed in *Cajanus cajan* seed extract when compared to leaf and root extracts. Phenolic compounds have redox properties which allow them to act as antioxidant (Mahitha *et al.*, 2015). As their free radical scavenging ability is facilitated by their OH groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity.

Total Flavonoids Content

The presence of Flavonoids content in analysed leaf, seed and root ethanolic extracts of *C.cajan* ranged as 36.17 mg\RE\gram, 49.43 mg\RE\gram, 48.32mg\RE\gram (Table 1). The highest content of Flavonoids was found in seed extracts while, leaf and root extracts have lowest Flavonoids content was assessed.

Antioxidant Assay

The antioxidant activity of ethanol extract of leaves, seeds and roots of *Cajanus cajan*

were investigated. The leaf, seed and root extract of *Cajanus cajan* was able to scavenge the free radicals in different concentration in a dose dependent manner in all the assays.

DPPH Assay

DPPH, relatively stable organic radical with a typical strong absorption band at 517nm invisible spectroscopy (deep violet colour) was used to evaluate the free radical scavenging capacity of the investigated samples. Ascorbic acid used as positive control for standard. Among the extracts tested, the seeds extract exhibited better

activity. The IC₅₀ values for leaves, seeds and roots extracts were 1.407, 0.844 and 1.184mg/ml respectively (Table 11). When the concentrations were lower the DPPH radical scavenging activity of leaves and roots extracts were weak, when the concentration was higher their activity much enhanced. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. Interestingly these values were significantly lower than the standard antioxidant tested. Our results are contradiction with Maneechai *et al.*, (2011).who reported that highest antioxidant activity was examined in ethanolic seed extracts by DPPH assay.

Table.1 Total Phenolic and Total Flavonoids content of *C.cajan* extracts

Different ethanolic extract	Total Phenols(mg of GAE\g of extract)	Total Flavonoids(mgQE\g extract)
Leaf	55.03	36.17
Seed	57.20	49.43
Root	50.95	48.32

Table.2 Invitro DPPH Scavenging activity of *C.cajan* leaf extract

DPPH Scavenging Activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	LEAF (%)	Ascorbic acid (%)
1	200	42.11	64.66
2	400	55.2	67.91
3	600	64.56	73.26
4	800	69.83	78.09

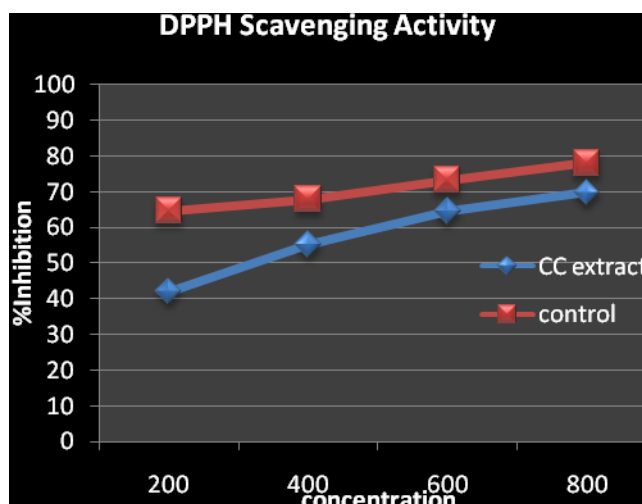


Table.3 Total antioxidant activity of *C.cajan* leaf extract

Total antioxidant activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	LEAF (%)	Ascorbic acid (%)
1	200	49.13	55.02
2	400	52.72	63.90
3	600	61.69	69.02
4	800	64.09	74.04

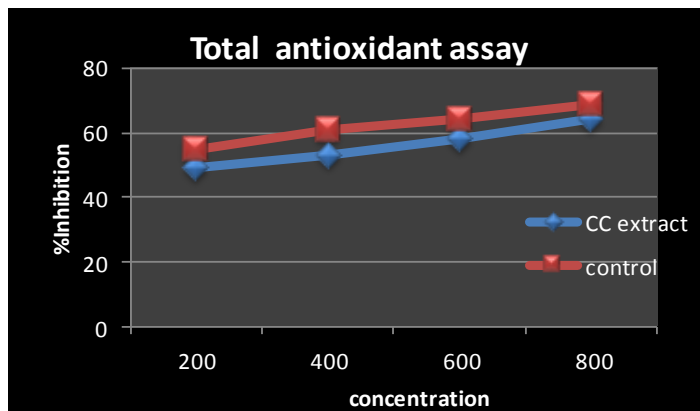


Table.4 H₂O₂ Radical assay of *C.cajan* leaf extract

H ₂ O ₂ Radical assay (% INHIBITION)			
S.NO	Concentration (µg/ml)	LEAF (%)	Ascorbic acid (%)
1	200	60.04	64.34
2	400	63.05	68.54
3	600	67.87	75.43
4	800	76.45	79.39

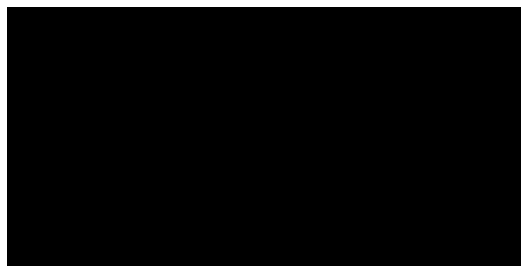


Table.5 Invitro dpph scavenging of *C.cajan* seed extract

DPPH scavenging activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	Cc Seed extract	Ascorbic acid
1.	200	46.6	52.82
2.	400	57.2	64.25
3.	600	67.8	74.78
4.	800	74.3	80.56

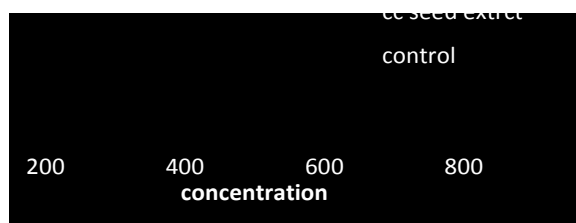


Table.6 Total antioxidant activity of *C.cajan* seed extract

Total antioxidant activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	Cc seed Extract	Ascorbic acid
1.	200	47.2	53.02
2.	400	54.6	59.12
3.	600	62.2	70.56
4.	800	70.2	74.5

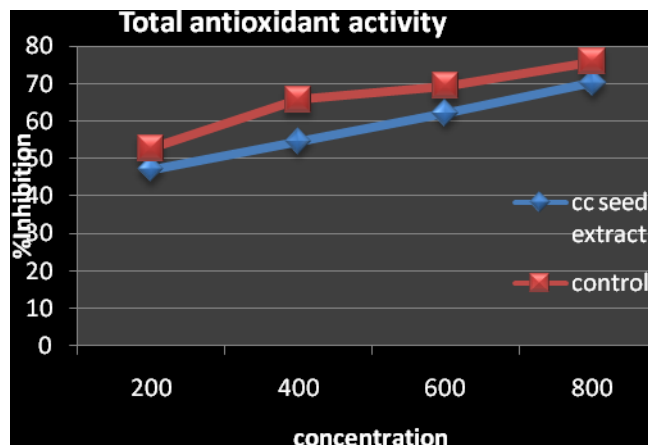


Table.7 H₂O₂ Radical assay of *C.cajan* seed extract

H ₂ O ₂ Radical assay (% INHIBITION)			
S.NO	Concentration (µg/ml)	Cc Seed extract	Ascorbic acid
1.	200	62.72	65.06
2.	400	66.32	69.89
3.	600	75.20	79.43
4.	800	79.07	83.98

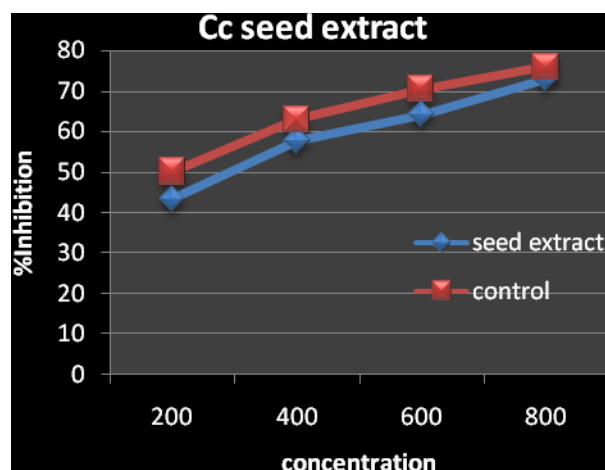


Table.8 Invitro DPPH scavenging activity of *C.cajan* root extract

DPPH Scavenging Activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	Cc root extract (%)	Ascorbic acid
1	200	43.07	49.90
2	400	56.08	60.45
3	600	63.65	69.45
4	800	71.48	76.89

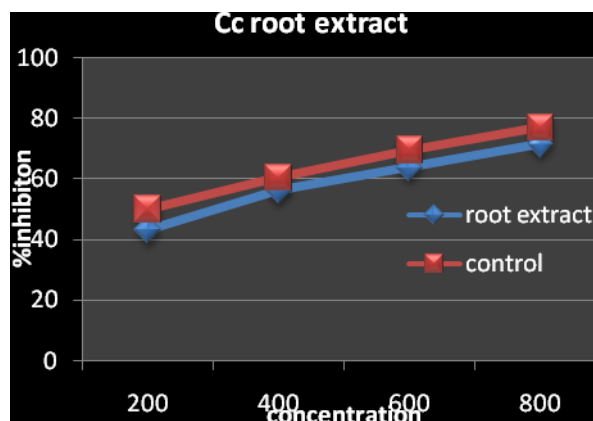


Table.9 Total antioxidant activity of *C.cajan* root extract

Total antioxidant activity (% INHIBITION)			
S.NO	Concentration (µg/ml)	Cc ROOT extract (%)	Ascorbic acid
1	200	45.53	53.98
2	400	52.82	58.56
3	600	58.82	67.93
4	800	69.32	75.86

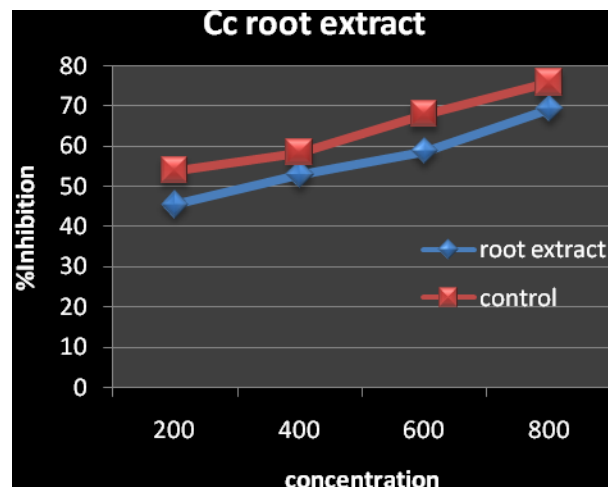


Table.10 H₂O₂ radical assays of *C.cajan* root extract

H ₂ O ₂ Radical assays (% INHIBITION)			
S. NO	Concentration (µg/ml)	Cc ROOT extract (%)	Ascorbic acid
1	200	59.99	64.71
2	400	65.05	69.01
3	600	72.12	75.90
4	800	75.21	80.40

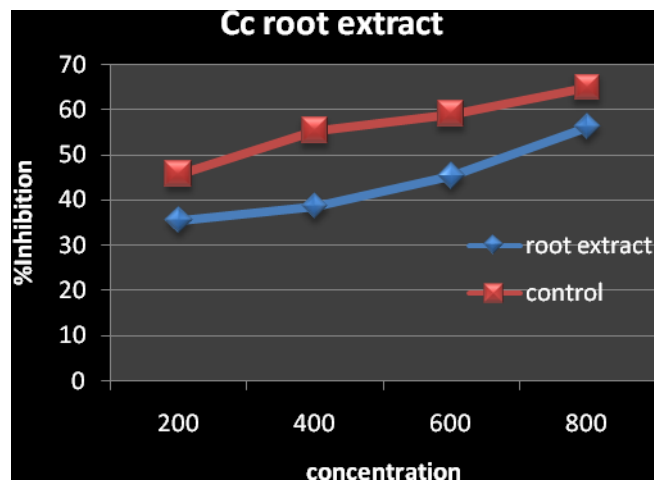


Table.11

IC ₅₀ for DPPH activity <i>Cajanus cajan</i>	
Leaf	1.407
seed	0.844
Root	1.184

Table.12

IC ₅₀ for H ₂ O ₂ activity <i>Cajanus cajan</i>	
Leaf	2.6
seed	2.4
Root	2.5

Total Antioxidant Capacity

To assess the total antioxidant capacity by phosphomolybdenum assay. The data on total antioxidant capacity observed in leaf, seed root extract of *Cajanus cajan* (fig). The antioxidant activity of phosphomolybdenum assay was found in the order seed>leaf>root. Among the extracts tested seed extracts showed highest antioxidant activity in comparison to others. The Result of the study are in conformity with the reports of Muhammad Jahangir *et al.*, (2014). They have reported that *Cajanus cajan* seeds exhibited the high antioxidant capacity in polar and non –polar fractions.

H₂O₂ scavenging activity

The hydroxyl radical scavenging activity of leaf, seed and root extracts of *Cajanus cajan* in various concentration (200,400,800 µg/ml) tested. The scavenging activity was found to be 62.72%, 66.32% 75.20% and 79.07% in seed and 60.04%, 63.05%, 67.87% and 76.45% in leaf and 59.99%, 65.05%, 72.12% and 75.21% in root extracts respectively. The IC₅₀ values of seed and leaf, root extracts were found to be 2.4 µg/ml, 2.6 µg/ml and 2.5µg/ml respectively (Table 12). In this activity ethanol extracts of *C.cajan* increased in dose dependent manner. Similarly, good hydroxyl radical scavenging activity was also reported in legume seed extracts. In the present study *C.cajan* seed extracts can be considered as good scavengers of hydroxyl radicals. H₂O₂ is relatively stable in the absence of reducing compounds. Scavenging of H₂O₂ by *Cajanus cajan* (leaf, seed, root) may be attributed for electrons donating abilities (wettasinge *et al.*, 2000).

In conclusion, the current study clearly stated that leaf, seed and root extract of *Cajanus cajan* posses a high amount of total

flavonoids as well as total phenolics making it a good source of natural antioxidants. The natural antioxidants that might be helpful in preventing various oxidative stress related disease, as ethanomedicine and also for creation of green drug commercially.

Acknowledgements

The project was supported by laboratory of Department of Microbiology & Biochemistry in Nadar Saraswathi College of Arts & Science, Theni. I thank the Management, Principal, Head of the Department, Faculty members for their guidance and support.

References

- Ahmad, I., Aqil, F., Mehmood, Z. 2006. antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk. J. Biol.*, 30: 177-183.
- Blois, M. 1958. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, 181: 1199-1200.
- Duker –Eshun, G., Jaroszewski, J.W., Asomaning, W.A., Oppong-Boachie, F. and Christensen, S.B. 2004. Antiplasmodial Constituents Of *Cajanus cajan*. *Phytother Res.*, 18: 128-130.
- Ghosh, T., Maity, T.K., Bose, A. 2009. *In vitro* free radical scavenging activity of ethanolic extract of leaves of *Cajanus Cajan* (L.)Mill sp. *J. Nat. Rem.*, 9(2):228-234.
- Grover, J.K., Yadav, S. and Vats, V.J. 2002. Medicinal Plants of India with Ant Diabetic Potential. *J. Ethnopharmacol.*, 81: 81-100
- King, Y., Fu, Y.J., Zu, Y.G., Chang, F.R., Chen, Y.H., Liu, X.L. 2010. Cajanus lactone A New Coumarin with Anti-Bacterial Activity from Pigeon Pea Leaves. *Food Chem.*, 121: 11505.
- Long, L.H., Chua, D.T.K., Halliwell, B. 2007. The antioxidant activities of seasonings

- used in Asian cooking. Powerful antioxidant activity of dark soy sauce revealed using the ABTS Assay. *Free Radical Biol. Med.*, 32: 181-186.
- Luo, Q.F., Sun, L., Si, J.Y., Chen, D.H. 2008. Hypocholesterolaemia Effect of Stilbenes Containing Extract Fraction from *Cajanus cajan* On Diet Induced Hypercholesterolemia In Mice. *Phytomed.*, 15: 932-9.
- Mahitha, B., Archana, P., Ebrahimzadeh, M.D.H., Srikanth, K., Rajinikanth, M., Ramaswamy, N. 2015. *In vitro* antioxidant and pharmacognostic studies of leaf extracts of *Cajanus cajan*(L.)Mill sp. *Indian Journal of Pharmaceutical Sciences*, 77: 170-177.
- Meenasahi, Devshreeverma and K.K. Harris. 2014. Phytochemical analysis Of The Leaf, Stem And Seed Extracts Of *Cajanus cajan* L(Dicotyledoneae: Fabaceae, *World J. Pharm. Pharma. Sci.*, Volume 3, Issue 8, 694-733.
- Mugammedjahagir, Raisakanum, Farhanamazhar. 2015. Antioxidant evolution of Polar and Non Polar Fractions of *Cajanus cajan* Seeds. *J. Med. Plants Res.*, Vol.9 (6), 193-198.
- Nneka, N., Vchegbu charies, N., Ishiwu 2016. Germinated pigeon pea (*Cajanus cajan*) a novel diet for lowering oxidative stress and hyperglycaemia. *Food sci. Nutrition*, volume 4, Issue 5.
- Pourmorad, F., Hosseinimehr, S.J., Shabimajd, N. 2006. antioxidant activity, phenols, flavonoids contents of selected Iranian medicinal plants. *S. Afr. J. Biotechnol.*, 5: 1142-1145.
- Prieto, P., Pineda, M., Aguilar, M. 1999. Spectroohotometric Quantification of Antioxidant Capacity through the Formation a Phosphomolybdenum Complex; *Specific Application of Vitamin E. Anal. Biochem.*, 269: 337-341.
- Raveena Devi, R., Premalatha, .R, Saranya, A. 2016. Comparative analysis of phytochemical constituents and antibacterial activity of leaf, seed and root extract of *Cajanus cajan* (L.)Mill sp *Int. J. Curr. Microbiol. App. Sci.*, 5(3): 485-494.
- Ruch, R.J., Cheng, S.J., Klauing, J.F. 1989. Prevention Of Cytotoxicity And Inhibition Of Intracellular Communication By Antioxidant Catechins Isolated From Chinesh Green Tea.*Carcinogenesis*, 10: 1003-1008.
- Wettasinge, M., Shahidi, F. 2000. Scavenging of reactive species and DPPH free radicals by extracts of borage and evening primrose meals. *Food chem.*, 70: 17-26.
- Wu, N., Fu, K., Fu, Y.J., Zu, Y.G., Chang, F.R., Chen, Y.H. 2009. Antioxidant Activities Of Extracts And Main Components Of Pigeon Pea Leaves. *Mol.*, 14: 1032-43.
- Zheng, Y.Y., Yang, J., Chen, D.H. and Sun, L. 2007. Effects of the stilbenes extracts from *Cajanus cajan* L. on ovariectomy-induced bone loss in rats. *Acta pharm. Sin.*, 42: 562-565.

How to cite this article:

Raveena Devi, R., R. Premalatha and Kayathri, R. 2016. Evaluation of Total Phenols, Total Flavonoids and *In vitro* Antioxidant Activity in the Ethanolic Leaf, Seed and Root Extract of *Cajanus cajan* (L.) Mill sp. *Int.J.Curr.Microbiol.App.Sci.* 5(10): 688-697.
doi: <http://dx.doi.org/10.20546/ijcmas.2016.510.075>