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Antioxidant evaluations of polar and non-polar fractions of *Cajanus cajan* seeds

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***Cajanus cajan* (L) Millsp., named Arhar in Hindi and Pigeon pea in English, is an important grain crop of rain-fed agriculture in semi-arid tropics, belonging to family Leguminosae. It is a food and a forage crop, rich in proteins with amino acids like methionine, lysine and tryptophan. Extensive studies on chemical composition of *C. cajan* have been done during last few decades. This article presents an overview of phytochemicals as a source of natural antioxidants in *C. cajan* seed extracts. The antioxidant potentials of the different fractions, that is, *n*-hexane, ethyl acetate, chloroform, methanol, *n*-butanol and aqueous, of *C. cajan* seeds were screened by using $ABTS^{\cdot+}$ assays, ferric reducing antioxidant power (FRAP) assay, total phenolic contents determination (TPC), total flavonoid contents (TFC) and metal chelating activity. Aqueous extract shows the highest trolox equivalent antioxidant capacity (TEAC) value, 140.69±0.34mM in $ABTS^{\cdot+}$ assays and the highest total phenolic content (TPC) value, 927.5±0.8 gallic acid equivalent (GAE). Hexane soluble fraction showed the highest percentage of metal chelating activity, that is, 79.0±0.5% bound iron and the highest FRAP value 49.08±0.55 g/ml, whereas *n*-butanol soluble fraction indicated the highest total flavonoid contents as 1691.1±0.2 mg/g QE.**

Key words: Leguminosae, *Cajanus cajan*, antioxidant potential, $ABTS^{\cdot+}$ assay, ferric reducing antioxidant power (FRAP) assay, total phenolic contents, total flavonoid contents, metal chelating activity.

INTRODUCTION

Human beings have been using plants for medicinal purposes throughout the history and even now are extensively using plant materials for preparing phyto-pharmaceuticals. Plants produce a vast array of secondary metabolites as a defense against environmental stress while same compounds can be used for pest control and healing wounds or injuries (Pal et al., 2006, 2007, 2011). The role of medicinal plants in disease prevention and control is due to the presence of antioxidant properties of their polyphenolic constituents, namely, flavones, flavonoids, isoflavones, anthocyanin, lignans, coumarin, catechins and isocatechins (Aqil et al., 2006) that are amphipathic in nature (Abbasi et al., 2010). These compounds are commonly found in both edible and nonedible plants and have multiple applications in food, cosmetics and pharmaceutical industries (Kahkonen et al., 1999). Free radicals such as hydroxyl radicals $(OH)^{\cdot}$ and superoxide anion $(O_2)^{\cdot-}$, reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl), are produced

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as normal products of cellular metabolism (Young and Woodsid, 2001). Mitochondrion is the chief site of metabolism. Rapid production of free radicals can lead to oxidative damage to biomolecules including proteins, lipids, purines, pyrimidine bases and sugar moiety of nucleic acid (Valko et al., 2007). Free radicals contribute to more than hundred disorders in human beings including atherosclerosis, arthritis and ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999).

Due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress cause free radicals depletion of immune system, change in expression and induce abnormal proteins (Halliwell, 1994). Synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to produce negative health effects. Moreover, these show low solubility hence their application is strongly restricted and there is a trend to substitute them with naturally occurring antioxidants (Branen, 1975).

This study was conducted to evaluate the antioxidant activities of *C. cajan* seed extract from different polarities. Because of the stability of radical cations under acidic, basic, neutral or in buffer solutions, assays are carried out under different reaction conditions. The early history of modern medicine contains description of plant derived phytochemicals, many of which are still in use (Pal et al., 2009, 2005; Mohammad et al., 2009).

C. cajan is a perennial plant, belonging to an important family Leguminoseae/Fabaceae. Other common names are red gram, Congo pea and no eye pea (Wu et al., 2009). The cultivation of Arhar goes back at least 3000 years. The center of origin is most likely Asia, from where it travelled to East Africa and then to America. This family is the third largest family amongst flowering plants. It is an erect, branched, hairy shrub, 1 to 2 m high. It is a multipurpose plant as its seeds are extensively used as dal. It is rich in proteins.

In India, its leaves are used for rearing silkworms, green pods are used as vegetable, husk. Green leaves and tops are used as fodder and green manure (Ambasta, 2004). Plants belonging to this family have high medicinal values. Infusion of leaves of *C. cajan* is used for curing anemia, hepatitis and diabetes, urinary infections and yellow fever (Kamboj, 2000). It is used as pain reliever in traditional Chinese medicine and also as sedative (Ahsan and Aslam, 2009).

In this article, we report the *in vitro* antioxidant capacities of organic fractions and aqueous extracts of *C. cajan*. In order to discover potential sources of natural antioxidants, various estimation methods like ABTS radical cation scavenging activity, FRAP assay, total phenolic contents (TPC) and total flavonoid contents (TFC) methods were followed as against conventionally used standards (Pall et al., 2011).

MATERIALS AND METHODS

Plant collection

Pigeon pea seeds were collected from the local market of Faisalabad, Pakistan in April, 2012 and were identified by Taxonomist at Department of Botany, GC University Lahore, Pakistan. Voucher specimen was deposited (Voucher No GC.Bot.Herb.1395) in the same place.

Extraction and fractionation of plant

2.0 kg of seeds were shade dried ground into fine powder and exhaustively extracted with methanol (2 L×3) at room temperature. Three extracts were mixed and concentrated to obtain greenish yellow semi-solid methanolic extract. It was dissolved in about (1.5 L) distilled water and treated with *n*-hexane (1.5 L) to yield non polar fraction along with chloroform (1.5 L), ethyl acetate (1.5 L), and *n*-butanol (1.5 L) to obtain polar fractions. Organic fractions and aqueous fraction was then concentrated on a rotary evaporator.

Chemicals and standards

2,4,6-tripyridyl-s-triazine (TPTZ), Trolox, gallic acid, Follin Ciocalteu reagent and butylated hydroxytoluene were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, sodium phosphate ammonium molybdate, ferric chloride and ferrous chloride from Merck (Pvt.) Ltd. (Germany).

Phytochemical analysis of the plant extracts

Qualitative tests for the investigation of phytochemical components like terpenes, phenols, saponins, flavonoids, alkaloids, tannins, sugars and cardiac glycosides in *C. cajan* were detected by using the methods reported by Sofowora, Trease and Evans (Prieto et al., 1999; Sofowora, 1996; Treas and Evans, 2009). The presence of a particular secondary metabolite was estimated from the intensity of color.

Test for terpenes (Salkowski test)

The presence of terpenes in *C. cajan* was confirmed by performing two tests. In the first test, spots of different extracts was placed on the TLC card and it was then sprayed with ceric sulphate solution followed by heating up to 105°C in order to develop the spots on a TLC plate heater. The presence of terpenes was indicated by the appearance of brown colored spots on the card. In the second test, 2 ml of chloroform were added to 0.5 g of each of the fraction in a test tube followed by careful addition of concentrated H_2SO_4. Appearance of reddish brown color suggested the presence of terpenes (Salkowski Test).

Tests for phenols

To 0.5 g of each of plant fraction, neutral solution of $FeCl_3$ was added; appearance of bluish-green color indicated the presence of phenols.

Tests for saponins

Saponins can be detected by vigorous shaking of 0.5 g of plant fraction with 0.5 ml distilled water; development of persistent froth

indicated the presence of saponins. Formation of an emulsion on shaking after the addition of few drops of olive oil further confirmed their presence.

Tests for flavonoids

Two methods were applied for the determination of flavonoids. In the first method, 10% of ammonia solution was added to 5.0 ml of each plant fraction followed by 1.0 ml of concentrated H_2SO_4. Appearance of yellow coloration which disappeared on standing indicated the presence of flavonoids. In the second method, appearance of persistent yellow color on addition of 1% aluminum chloride to different plant fractions indicated the presence of flavonoids.

Tests for alkaloids

Alkaloids were tested for their presence by conventional methods. Spots of different fractions were placed on a piece of TLC card followed by drying and spraying with Dragon Dorff's reagent. Development of reddish-brown color indicated the presence of alkaloid. Also addition of Mayer's reagent to a small portion of each fraction, resulted in cream colour precipitates, confirmed the presence of alkaloids.

Test for tannins

Tannins were indicated by boiling 0.5 g of each fraction in 10 ml of distilled water. As after filtration few drops of 1% $FeCl_3$ solution were added and appearance of brownish-green or bluish-black coloration confirmed the presence of tannins.

Tests for reducing sugars

To 0.5 ml of the sample fraction, 0.5 ml of Fehling solution A and B were added; reaction mixture was strongly heated for about 1 min. Appearance of the red precipitates confirmed the presence of reducing sugars.

Tests for cardiac glycosides

A violet or brown colored ring formation showed the presence of cardiac glycosides when 1 ml of glacial acetic acid was added along with a drop of $FeCl_3$ followed by the addition of 1.0 ml of concentrated sulphuric acid.

Antioxidant assays

The following assays were performed to determine the antioxidant activity. Results were recorded as shown in Table 1.

$ABTS^+$ Assay

Total antioxidant activity was measured in terms of Trolox Equivalent Antioxidant capacity (TEAC) method described by Re et al. (1999). 7 mM solution of ABTS was prepared in double distilled water with 2.45 mM potassium persulphate to generate $ABTS^+$. $ABTS^+$ solution was diluted with TBS buffer at pH 7.4 to obtain an absorbance of 0.70±0.02 at 734 nm. 10 µl of sample was added to 2.29 ml of diluted solution of $ABTS^+$. Absorbance reading was taken exactly after 1 min of initial mixing up to 6 min at 30°C. The percentage inhibition of absorbance at 734 nm was determined using the formula:

Age inhibition (at 734nm) (%) = $(1-A_f/A^o) \times 100$

A^o is the absorbance of ABTS radical cation and A_f is the absorbance after sample addition.

Ferric reducing antioxidant power

Twenty five milliliters of 300 mM acetate buffer with pH 3.5 and 2.5 ml of 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ (2.5 ml) were mixed to prepare the FRAP reagent. To 100 ml of each sample, 3 ml of FRAP reagent and 300 µl of distilled water was added and the absorbance was taken at 593 nm. FRAP values were calculated from standard curve of Iron (II) Sulphate (Benzie and Strain, 1996).

Total phenolics contents

Forty microliters from each of the sample were mixed with 3.16 ml of distilled water and 200 µl of 0.2 N Follin Ciocalteu reagent. 600 µl of super saturated sodium carbonate (75 g/L) was added and solution turned blue after 8 min. Absorbance was measured at 765 nm after incubation with intermittent shaking at 40°C for 30 min. Total phenolic contents were calculated from the standard curve expressed as mg/g equivalents of gallic acid (GAE) (Slinkard and Singleton, 1997).

Total flavonoid content determinations

TFC contents of various fractions were determined by the standard method (Dewanto et al., 2002). 0.25 ml of plant sample and quercetin standard solution was mixed with 1250 µl of distilled water in a test tube with addition of 75 µl of $NaNO_2$ (0.5 M), after 5 min. 0.5 ml of 1 M NaOH solution was added and volume was raised to 2.5 ml by adding distilled water. Absorbance was measured at 510 nm. TFC contents were determined from the standard curve expressed as milligrams of quercetein equivalents per gram of sample.

Metal chelating activity

The reaction mixture was prepared by adding 100 ml of sample in 0.05 ml of 2.0 mM $FeSO_4$ and 0.2 ml of 5 mM ferrozine and total volume was made up to 4 ml with double distilled ethanol, after 10 min absorbance was noted at 562 nm (Dinis et al., 1994). Percentage of bound iron was calculated using the formula in terms of EDTA standard.

Bound iron (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

RESULTS

Phytochemical screening to find out the biologically active constituents confirmed the presence of terpenoids, phenols, saponins, flavonoids, alkaloids and reducing sugars. Results revealed that chloroform, ethyl acetate, *n*-butanol, *n*-hexane soluble fractions, methanolic and aqueous extracts were rich in terpenes, phenolics, flavonoids, saponins and reducing sugars with traces of

Table 1. Phytochemical screening results of *Cajanus cajan*.

Phytochemical	Methanolic fraction	n-hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*-butanol fraction	Aqueous fraction
Terpenes	++++	++	+++	+++	+++	++++
Phenols	+++	++	+++	+	+++	++++
Saponins	++	+++	++	+	+	-
Flavonoids	++	+++	+++	+++	++++	+
Alkaloids	+	++	+++	-	-	-
Tannins	+	+++	-	-	+++	-
Red. Sugrs	-	+	+++	++++	+	++
Card.glyc	-	-	-	-	-	-

Red. Sugrs = Reducing sugars, Card. glyc = Cardiac glycosides, Fr. = Fraction. ++++ Very strong; +++ Strong; ++ Medium; + Poor presence; - Absence. Experiments repeated three times for each treatment. Classification was based on observation of color intensity and amount of precipitates.

Table 2. Antioxidant potential of different fractions.

Plant sample	ABTS	FRAP value TE	TPC	FC	Metal chelating activity
	(TEAC M) ±S.E.M.	(µM/mL) ±S.E.M.	(GAE mg/g of extract) ± S.E.M.	(mg/g QE) ± S.E.M.	Bound iron (%)
Crude methanolic extract	109.07±0.2	49.08±0.5	510.12±0.2	968.27±0.1	79.00
n-hexane soluble fraction	89.54±0.4	11.92±0.2	320.01±0.1	1075.50±0.7	44.30
Chloroform soluble fraction	109.00±0.3	19.12±0.1	475.0±0.3	1441.0 ±0.3	09.26
Ethyl acetate soluble fraction	89.52±0.3	16.60±0.5	282.50±0.6	1387.50±0.2	15.46
n-butanol soluble fraction	103.00±0.1	09.08±0.2	430.80±0.4	1691.10±0.2	85.40
Aqueous extract	140.69±0.3	44.08±0.1	927.50±0.8	252.80±0.4	15.86

tannins. Considerable amounts of alkaloids were detected in chloroform fraction but cardiac glycosides were totally absent. Crude methanolic extract was found to contain tannins, flavonoids, terpenoids, phenols, saponins and alkaloids. Aqueous fraction contained good amounts of terpenoids and phenols along with reducing sugars, but lacked in tannins, saponins, alkaloids and cardiac glycosides.

Antioxidant evaluations were performed by using the conventional methods. $ABTS^{+}$ scavenging activities assay was calibrated by using an alpha Tocopherol analog 'Trolox' as standard. The results of $ABTS^{+}$ were expressed in terms of TEAC values as shown in Table 2. TEAC is a measure of effective antioxidant activity of the substance and stands for "Trolox equivalent antioxidant capacity". High TEAC value representing high $ABTS^{+}$ scavenging shows a greater antioxidant potential of the sample. Aqueous fraction showed the highest activity with methanolic and chloroform fractions showing slightly less activity. *n*-butanol fraction indicated greater activities and *n*-hexane and ethyl acetate fractions showed moderate activities. The TEAC values showed the following trend: aqueous extract > methanol > chloroform > *n*-butanol > ethyl acetate = *n*-hexane.

Ferric reducing antioxidant power assay involved the reduction of ferric tripyridyltriazine Fe(III)-$(TPTZ)_2Cl_3$ (pale yellow in color) to blue coloured ferrous Fe (II)-$(TPTZ)_2Cl_2$ complex at 593 nm absorbance on reacting with an antioxidant. Results were expressed in FRAP units. A higher value referred to a high reducing power as shown in Table 2. The absorbance reading was taken just 6 min interval after mixing TPTZ to the sample. FRAP value was the highest for aqueous extract, slightly less in ethyl acetate fraction but *n*-hexane and chloroform fraction showed moderate activities. Phenolic contents of the plant materials were suggested to correlate directly with antioxidant activities and also play an important role in stabilizing lipid oxidation. Order of reactivity is found to be methanol > aqueous fraction > chloroform > ethyl acetate > *n*-hexane > *n*-butanol.

The aqueous fraction was found to have the highest phenolic contents while ethyl acetate extract had the lowest phenolic contents relative to butylated hydroxy-toluene taken as a reference standard having total antioxidant activity 0.928±0.09. The most powerful antioxidants are phenolic compounds having hydroxyl group in their structures and they can be detected by TPC assay. Results as shown in Table 2 represent the

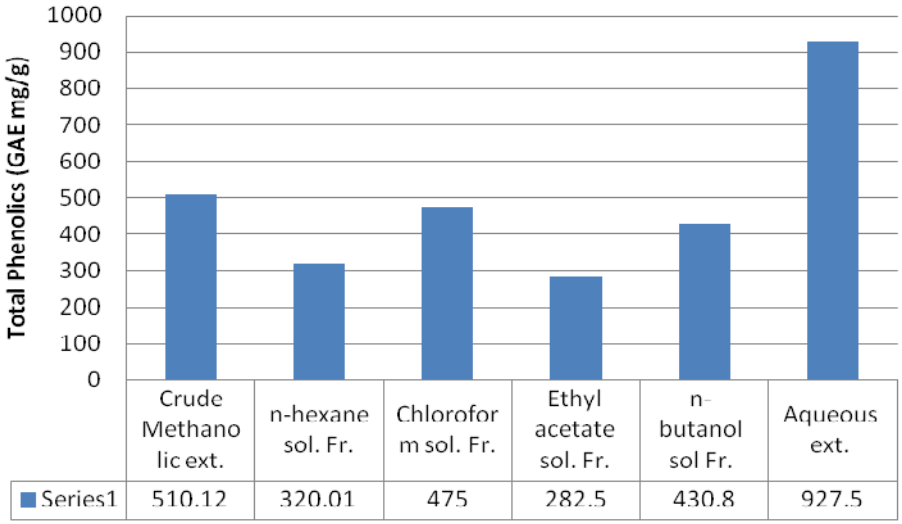

Figure 1. Total Phenolic Contents in *C. cajan*. Ext.: Extract; sol.: Soluble; Fr.: Fraction.

total phenolics in different fractions found in the order: aqueous fraction > methanolic extract > chloroform > *n*-butanol > *n*- hexane > ethyl acetate.

The principle for the determination of total flavonoids is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-4 or C-5 hydoxyl group of flavones or flavonoles. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl group in the A- or B-ring of flavonoids with peak absorption at 400 nm. Table 2 represents the total flavonoid contents and observed order of reactivity of fractions: *n*-butanol > chloroform > ethyl acetate > *n*-hexane > methanolic extract > aqueous fraction.

Metal ion chelating activity results were expressed in percentage of bound iron. Iron (II) formed a colored complex with ferrozine which can be determined at 562 nm. *n*-butanol fraction had shown a maximum value of percentage bound iron. The other fractions show activity in order *n*-butanol > methanolic extract > n-hexane > aqueous extract > ethyl acetate > chloroform.

DISCUSSION

Results of ABTŚ+ assays were expressed in terms of TEAC values, that is, measure of effective antioxidant activity of a substance expressed as Trolox equivalent antioxidant capacity (Dinis et al., 1994). ABTŚ+ assays result revealed that aqueous extract and chloroform fraction of *C. cajan* has good antioxidant activity having 140.69 and 109.07 TEAC, respectively. Both extracts are rich in phenol concentration but maximum in aqueous extract, while chloroform extract contain fair amount of flavonoids and saponins as well and probably responsible for these activities.

FRAP assays results are significant in case of methanolic extract 49.08µM/ml and aqueous extract 44.08 µM/ml and insignificant for n-hexane fraction 11.92 µM/ml. Methanolic and aqueous extracts of the plant under consideration are equally important. Total phenolic contents are suggested to correlate directly with antioxidant activities as polyphenolic compounds play an important role in stabilizing lipid peroxidation. Phenolic compounds are the powerful antioxidants having hydroxyl group in their structures (Ivanova et al., 2005). On the basis of results presented in Table 2, total phenolic contents are excellent in aqueous fraction of the said plant material having a value of 927.5±0.8 GAE mg/g. Results are also expressed in Figure 1. So the seeds of *C. cajan* are a good source of phenolic compounds.

Flavonoids are recognized as potent antioxidants due to their phenolic hydroxyl group constitution. They can delay or inhibit oxidation process of lipids by inhibiting the initiation or propagation of oxidative chain reactions known as primary antioxidants. Total flavonoid contents of different polar and non-polar fractions ranges from 1691 to 1075 for n-butanol and n-hexane fractions, respectively. These results are an evidence for a good yield of total flavonoid contents of the seeds of *C. cajan*.

Iron is known to generate free radicals through the Fenton and Haber-Weiss reaction. Metal ion chelating activity of an antioxidant molecule prevents oxy-radical generation. As a result, oxidative damage also reduces the concentration of the catalyzing transition metal. High percentage of bound iron exhibited by n-butanolic fraction and methanolic extracts of *C. cajan* seeds suggested the high antioxidant potentials.

Conclusion

Screening of polar and nonpolar seed fractions of *C. cajan* revealed the presence of a beautiful aggregation of phytochemicals which are responsible for the importance

of this plant as a good source of natural antioxidants.

It was concluded that ethyl acetate soluble fraction, methanolic and aqueous extracts are rich in terpenes, phenols, flavonoids, tannins, saponins and cardiac glycosides. Ethyl acetate soluble fraction showed good activity due to the presence of many bioactive compounds whereas methanol soluble fraction showed moderate to good activity showing that these fractions are also valuable sources of bioactive compounds as well as good antioxidants. Alkaloids are also present in appreciable amounts and extracted.

Oxidative stress is understood a major cause of developing a number of diseases like Alzheimer's disease (Nunomura et al., 2006), Parkinson's disease (Wood-Kaczmar et al., 2006), rheumatoid arthritis (Hitchon and El-Gabalawy, 2004) and neuro degenaration in motor neuron disease (Cookson and Shaw, 1999). Fortunately, seeds are the edible part of plant, it is the easiest and cheapest way to obtain the natural antioxidants by taking them as a dietary component. Due to the presence of antioxidants properties in their constituents, this plant may play an important role in disease prevention and control without any side effects and toxicity as compared to synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters have been suspected to produce negative health effects (Branen, 1975). Moreover, low solubility of synthetic antioxidants restricted their application. The use of Pigeon pea seeds in our food plan may play an important role in disease prevention and control because of its valuable antioxidants properties.

Conflict of Interest

Authors have not declared any conflict of interest.

REFERENCES

Abbasi MA, Zafar A, Riaz T, Aziz-ur-Rehman, Arshad S, Shahwar D, Jahangir M, Siddiqui SZ, Shahzadi T, Ajaib M (2010). Evaluation of comparative antioxidant potential of aqueous and organic fractions of *Ipomoea carnea*. J. Med. Plants Res. 4:1883-1887.

Ahsan R, Islam M (2009). *In vitro* antibacterial screening and toxicological study of some useful plants (*Cajanus cajan*). Eur. J. Sci. Res. 41:227-32.

Ambasta SP (2004). The useful plants of India. National Institute of Science Communication. 4th ed. New Delhi, India. pp. 94-5.

Aqil F, Ahmed I, Mehmood Z (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 30:177-183.

Benzie IFF, Strain JJ (1996). The reducing ability of plasma as a measure of antioxidant power-the FRAP assay. Anal. Biochem. 239:70-76.

Branen AL (1975). Toxicology and biochemistry of butylated hydroxyanisole and butylated hydoxytoluene. J. Am. Oil Chem. Soc. 52:59-63.

Cookson M, Shaw P (1999). Oxidative stress and motor neurone disease. Brain Pathol. 9(1):165-86.

Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J. Agric. Food Chem. 50(10):3010-4.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315:161-169.

Evans WC (2009). Trease and Evans Pharmacognosy. Elsevier Limited. Edn. P 16.

Halliwell B (1994). Free radicals, antioxidants and human disease: curiosity, cause or consequences? Lancet 344(8924):721-724.

Hitchon CA, El-Gabalawy HS (2004). Oxidation in rheumatoid arthritis. Arthritis Res. Ther. 6:265-78.

Ivanova D, Gerova D, Chervenkov T, Yankova T (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J. Ethnopharmacol. 96(1-2):145-150.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47(10):3954-62.

Kamboj VP (2000). Herbal medicine. Curr. Sci. 78(1):35-39.

Kumpulainen JT, Salonen JT (1999). Natural antioxidants and anticarcinogens in nutrition, health and disease. Royal Soc. Chem. 178-187.

Mohammad R, Jahan MI, Fahmidul HA, Haque M (2009). An ethnobotanical survey and pharmacological evaluation of medicinal plants used by the Garo tribal community living in netrakona district Bangladesh. Adv. Nat. Appl. Sci. 3:402-418.

Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA (2006). Involvement of oxidative stress in Alzheimer disease. J. Neuropathol. Exp. Neurol. 65(7):631-41.

Pal D, Sarkar A, Gain S, Jana S, Mandal S (2011). CNS depressant activities of *Coccos nucifera* in mice. Acta. Pol. Pharm. 68(2):249-54.

Pal DK, Mandal M, Senthilkumar GP, Padhiari A (2006). Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. Fitoterapia 77(7-8):589-91.

Pal DK, Pahari SK, Pathak AK (2007). Evaluation of CNS activities of aerial parts of *Jasminum multiflorum* Andr. Asian J. Chem. 19:4452-8.

Pal DK, Sahoo M, Mishra A (2005). Analgesic and anticonvulsant effects of saponin isolated from the stems of *Opuntia vulgaris* Mill in mice. Eur. Bull. Drug Res. 13:91-7.

Pal DK, Sannigrahi S, Mazumder UK (2009). Analgesic and anticonvulsant effects of saponin isolated from the leaves of *Clerodendrum infortunatum* Linn. in mice. Indian J. Exp. Biol. 47:743-7.

Pall D, Mishra P, Sachan N, Ghosh AK (2011). Biological activities and medicinal properties of Cajanus cajan (L) Millsp. J. Adv. Pharm Technol. Res. 2(4):207-214.

Prieto L, Pineda M, Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269:337-341.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9-10):1231-7.

Slinkard K, Singleton VL (1997). Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Vitc. 28:49-55.

Sofowora A (1996). Research on medicinal plants and traditional medicine in Africa. J. Alt. Comp. Med. 2(3):363-372.

Valko M, Leibfritz D, Moncola J, Cronin MT, Mazur M, Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell. Biol. 39(1):44-84.

Wood-Kaczmar A, Gandhi S, Wood NW (2006). Understanding the molecular causes of Parkinson's disease. Trends Mol. Med. 12(11):521-8.

Wu N, Fu K, Fu YJ, Zu YG, Chang FR, Chen YH, Liu XL, Kong Y, Liu W, Gu CB (2009). Antioxidant activities of extracts and main components of pigeonpea Cajanus cajan (L.) Millsp. leaves. Molecule. 14(3):1032-43.

Young IS, Woodside JV (2001). Antioxidants in health and disease. J. Clin. Pat. 54:176-186.