Assessment of total phenolic content and antioxidant potential of methanol extract of *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne.

Madiha Khan[1], Ghazala H Rizwani[1], Huma Shareef[1]*, Sanja Ćavar[2] and M Zia-Ul-Haq[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan
[2]Faculty of Science, University of Sarajevo, Sarajevo-71000, Bosnia and Herzegovina

Abstract: Plants are rich in a variety of chemical compounds. Many are secondary metabolites including aromatic substances most of them are phenols or their oxygen substituted derivatives. Medicinal plants are also rich in antioxidant constituents such as phenols, tocopherols, ascorbic acid, carotenoids, and flavonoids etc. They are found to acquire free radical scavenging activity and used worldwide for the treatment of various immune system dependent diseases. *Peltophorum pterocarpum* (DC) Backer ex K. Heyne (*Caesalpiniaceae*) is a beautiful ornamental tree, widely grown in tropical regions and its parts are used in traditional medicine as an effective therapeutic agent.
Fresh pods of *Peltophorum pterocarpum* was evaluated for its antioxidant potential by using various methods including DPPH, superoxide anion, nitric oxide scavenging, and metal chelating activity. TPC via Folin-Ciocalteau's reagent and anti haemolytic activity red blood cells respectively have also been measured. The methanol extract of pods of *Peltophorum pterocarpum* was found to possess the significant amount 439.21±0.17 mg GAE (gallic acid equivalents) / g of TPC. The antioxidant potential of pods extract at mature stage showed potent activity and measured as, free radical scavenging activity 73.29±0.81%, superoxide anion scavenging activity 89.03±1.07%, nitric oxide scavenging activity 84.25±1.18%, and metal chelating activity 64.12±0.11%. The extract also showed potent anti haemolytic activity 79.09± 0.75%. *Peltophorum pterocarpum* exhibited strong but varying level of antioxidant and anti haemolytic activity in various methods along with total phenolic contents.

Keywords: Antioxidant activity, DPPH, *Peltophorum pterocarpum*, Phenolic content.

INTRODUCTION

Plant source is the precursor of many natural products and secondary metabolites and are being used in the traditional system or as an alternative medicine for treatment of diseases, which is gaining momentum, also in western countries. They have exposed great prospective in take care of life threatening ailments for example many infectious diseases, cancer, heart diseases, and diabetes (Lai *et al.,* 2010; Derjani *et al.,* 2011). Secondary metabolic products are vital for plant defense mechanism. On the basis of their origin, they are classified into several types of phytochemicals i.e. alkaloids, flavonoids, phenols, steroids, glycosides and terpenes, etc. They possess antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties (Yen *et al.,* 1993). These phytochemical agents having therapeutic activities are utilized in drug development either as a single therapeutic agent or in combination with other agents.

Phenolic compounds are one of the chief phytochemicals which are established universally as antioxidants or free radical scavengers and act as reducing agents, metal chelators and singlet oxygen quenchers (Chew *et al.,* 2009). They play an essential role in management of many health problems such as cancer, rheumatoid arthritis, cardiovascular, Alzheimer's, neurodegenerative disorders and aging. Phenolics were proved to be beneficial for the above disorders as they scavenge free radicals though preventing destructive effects to the cell proteins, lipids and carbohydrates (Barry, 1991). Besides the above, the demand of phenolic compounds is increased in the food industry, in terms of application as well as in the restricted use of synthetic antioxidants. Artificial antioxidants such as BHA or BHT are consumed to inhibit reactive oxygen species (ROS) which cause DNA damages. But, they are unstable and highly volatile in character so their safety and efficacy were an issue but the phenolics delayed oxidative degradation of lipids and thereby acquire the better quality and nutritional value of food (Kähkönen *et al.,* 1999). As a result, there is a need to look for substitutes to synthetic antioxidants from the natural and safe sources especially from plant origin.

Peltophorum pterocarpum (DC) Backer ex K. Heyne, belongs to the family *Caesalpiniaceae*, commonly called as Copper Pod and Rusty Shield Bearer. It is widely grown in tropical regions including South Eastern Asia as an ornamental tree as well as in Pakistan as a normal flora. A handsome tree, up to 24 m. in height, bark grey, smooth; leaves bipinnate: leaflets many, small, obliquely oblong, deep green; flowers in large, erect, terminal

*Corresponding author: e-mail: phr_huma@hotmail.com

panicles, yellow, fragrant; pods flat, winged, 5-10cm. Long, reddish brown; seeds 1-5, oblong, lattened brownish. The bark which contains 20.8% of catechol type of tannins and 9.5% non-tans, can be used for tanning purpose. The heart wood is red, hard and strong and is durable when protected from weather. It is suitable for planks, coach building, furniture and cabinet work. Leaves are rich in proteins (54.7%) and can be used as a cattle feed. Leaf protein has specific amino acid composition: arginine, 5.8; histidine, 1.9; lysine, 5.4; tyrosine, 5.2; tryptophan, 2.0; phenylalanine, 5.5; cystine, 1.5; methionine, 2.0; threonine, 4.9; and vaniline 7.0 gm/16 gm N (Anon., 1966). In conventional remedy, flowers are used as an astringent to heal or relieve intestinal disorders, childbirth pain, sprains, bruises and swelling. Its lotion is employed in eye troubles, muscular pains and sores (Sethuraman *et al.,* 1984). Moreover it is also utilized for gargles and as tooth powders. The flowers and bark are also reported to have antimicrobial activity (Duraipandayan *et al.,* 2006; Valdlapudi *et al.,* 2010). The leaf and bark of this plant reported to contain phenolic compounds that showed antibacterial, antioxidant and hypoglycemic activity (Jagessar *et al.,* 2008; Jain *et al.,* 2011; Ling *et al.,* 2010; Yafang *et al.,* 2011).

Aim of the study was to investigate *Peltophorum pterocarpum* (DC) Backer ex K. Heyne pods extract for its antioxidant and antihaemolytic potential.

MATERIAL AND METHODS

Plant collection

Fresh pods of *Peltophorum pterocarpum* were collected during the month of June 2011 and identified by a taxonomist Dr. Anjum Perveen, Department of Botany, University of Karachi. A voucher specimen number 086 has been deposited in herbal museum of Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

Preparation of extract

Fresh pods of *Peltophorum pterocarpum* (500gm) were collected, garbled and chopped into small pieces, then percolated in 2.5 L of 80% methanol (Merck) for 7-8 days at room temperature. The percolate was filtered off through a filter paper Whatman No.1. The procedure was repeated thrice and volume of percolate was reduced volume through evaporation rotary evaporator (BÜCHI Rotavapor R-200 Switzerland) at controlled temperature (40°C) and reduced pressure. The concentrated methanolic extract was lyophilized by freeze dryer (EYELA freeze dryer FD-1 Tokyo Rikakikai Co. Ltd) for the complete removal of solvent. The freeze dried methanolic extract 21.0g of *Peltophorum pterocarpum* was stored in an air tight container at room temperature.

Chemicals

Methanol, catechin, gallic acid, quercitin, Folin Ciocalteu's reagent, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$), trichloroacetic acid (TCA), thiobarbituric acid (TBA), butylated hydroxyl toluene (BHT), butylated hydroxyanisole (BHA), L-Ascorbic acid and 3-(2-pyridyl)-5,6- bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals and solvents were of Analytical Dual-beam UV-VIS spectrophotometer (Uvikon XL, Bio-Tek Instruments, Bad Friedrichshall, Germany) was used for all determinations.

Determination of Total phenolic content (TPC)

Total phenolic content was determined by using Folin-Ciocalteu's reagent method (Sharma *et al.,* 2011). Briefly, extract (200 µl) was oxidized with Folin-Ciocalteu reagent (1 ml; 0.5 N), reaction was neutralized with 1 ml Sodium carbonate (75 g/L) and incubated for 2 hrs at room temperature. TPC was measured at 760 nm on Dual-beam UV-VIS spectrophotometer. Quantification of TPC was done on the basis of the standard curve of gallic acid and results were expressed as milligram of gallic acid equivalent (mg GAE/g).

DPPH free radical scavenging activity

Free radical scavenging assay was performed by following method (Naim *et al.,* 1976). Stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493%. Stock solution (5 ml) was added to 1 ml of extract solution (500µg/ml). After 30 min, absorbance was measured at 517 nm spectrophotometrically and concentration was calculated by standard calibration curve. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Ao - A}_1}{\text{Ao}} \times 100$$

where A_o = absorbance of the control and A_1= absorbance of the extract. Scavenging activity was compared with standards like ascorbic acid, α-tocopherol, BHA and BHT ran simultaneously.

Superoxide anion radical scavenging assay

For measurement of anion radical scavenging potential, reaction mixture included nitro blue tetrazolium (pH 7.4; 1 ml; 156 mM), NADH solution (pH 7.4; 1 ml; 468 mM) and extract (500 µg/ml). To initiate the reaction, 100 ml of 60 mM phenazine methosulfate (pH 7.4) was added to the mixture and incubated at 25°C for 5 min. Absorbance was measured at 560 nm against blank and was compared with standards. Decreased absorbance of reaction mixture was indicative of increased superoxide anion scavenging activity. Following formula was used to calculate % inhibition of superoxide anion generation:

$$\text{Inhibition } (\%) = \frac{Ao - A_1}{Ao} \times 100$$

where A_o = absorbance of the control and A_1 = absorbance of the extract (Ilhami *et al.,* 2005). BHA and α-tocopherol were used for comparison.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan *et al.,* 2003) by mixing sodium nitroprusside (5 mM) dissolved in phosphate buffered saline with methanolic extract (500 µg/ml). After ambient incubation for 30 min, 1.5 ml of this solution was taken and diluted with 1.5 ml of Griess reagent (prepared by dissolving 1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). During reaction, diazotization of nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride resulted in the formation of active chromophore that was estimated at 546 nm then the measured percent scavenging activity was compared with ascorbic acid which was used as standard.

Metal chelating activity

Chelating power of the methanol extract for ferrous ions was measured following a previously reported method (Sabate, 2003). The extract (0.5 ml), deionised water (1.6 ml) and $FeCl_2$ (0.05 ml; 2 mM) were mixed by vortex mixer. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with divalent iron to form stable water-soluble magenta complex species. After 10 min, absorbance of Fe^{2+}–Ferrozine complex was measured spectrophotometerically at 562 nm. Blank were also performed for the comparison.

Antihaemolytic assay

Antihaemolytic activity of methanolic extract was performed according to method of (Manaharan *et al.,* 2011), whereby Erythrocytes (red blood cells) obtained from cow's blood which was diluted with phosphate buffered saline to prepare 4% suspension. MeOH extract (500 µg/ml saline buffer) was added to 2 ml of this suspension and the volume was made up to 5 ml with saline buffer. After incubating the mixture at ambient conditions for 5 min, 0.5 ml of H_2O_2 solution of appropriate concentration in saline buffer was added to mixture to induce oxidative degradation in the lipoprotein membrane. The concentration of H_2O_2 in the reaction mixture was adjusted to bring approximately 90% haemolysis in blood cells. The reaction mixture was then centrifuged at 800 × g for 10 min and extent of haemolysis was determined by measuring the absorbance at 540 nm to measure haemoglobin liberation. Percent inhibitory activity of extract on haemolysis was calculated and compared with α-tocopherol and BHA.

STATISTICAL ANALYSIS

All the analyses were performed in triplicate to minimize the incidence of error. "MSTATC" statistical computer package was used during the analysis of variance and LSD calculations.

RESULTS

Total phenol content

Total phenol contents of *Peltophorum pterocarpum* methanol extract of pods, as determined by Folin Ciocalteu's reagent, are reported as mg of gallic acid equivalent and found to be 439.21±0.17 mg/g of extract as shown in table 1.

Table 1: Total phenol content (mg GAE/g) of *Peltophorum pterocarpum* extract.

Sample	TPC (mg GAE/g)
Peltophorum pterocarpum extract	439.21 ± 0.17

Data are expressed as means ± standard deviations (n = 3) on dry weight basis.

DPPH radical-scavenging activity

The radical-scavenging activity of the extract was found to be 73.2±0.81% while the values for ascorbic acid, BHT, BHA and α-Tocopherol were 67.47±0.09%, 61.31±1.25%, 54.11±1.95% and 67.26±0.22% respectively, as shown in table 2.

Superoxide anion scavenging activity

The extract showed 89.03±1.07 % superoxide scavenging activity where as the percentage inhibitions for standards ascorbic acid, BHA and α-Tocopherol were determined as 92.22±0.40%, 73.36±1.31% and 78.16±1.14% respectively as shown in table 2.

Nitric oxide-scavenging activity

Extract of *Peltophorum pterocarpum* showed good NO scavenging activity i.e., 84.28±1.18% compared with ascorbic acid as standard as shown in table 2.

Metal chelating activity

Extract showed good iron chelating ability i.e., 64.12±0.11a % mentioned in table 2.

Antihaemolytic activity

The extract did not affect the erythrocytes harmfully and, in fact, exhibited potent antihaemolytic activity i.e., 79.09±0.75% compared with BHA and α-Tocopherol which served as a positive control with 82.12±0.13% and 74.43±0.39% respectively as shown in table 2.

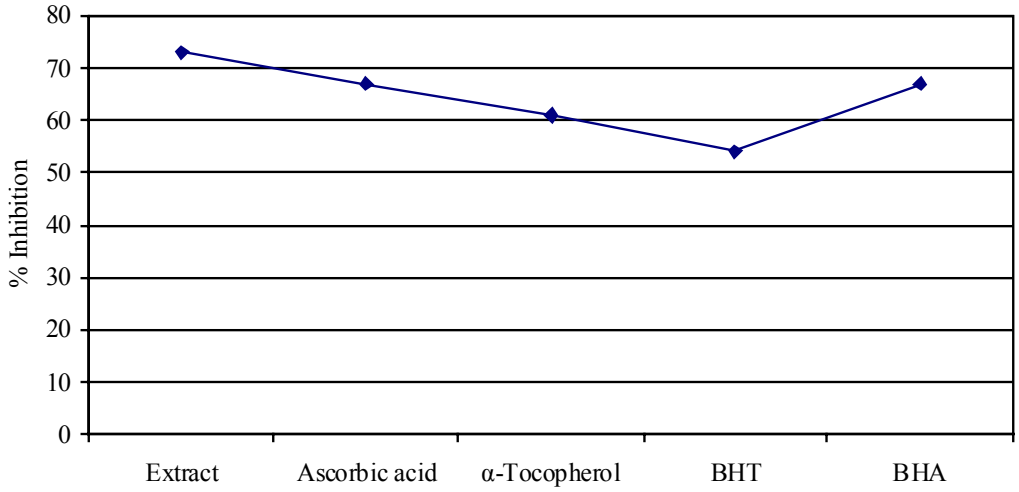

Fig. 1: DPPH Radical scavenging activity of the extract compared with standards.
Extract concentration: 500 µg/ml, BHT: butylhydroxytoluene, BHA: butylhydroxyanisole

Table 2: *In vitro* antioxidant activity (% inhibition) of *Peltophorum pterocarpum* extract

Sample	DPPH radical scavenging activity	Superoxide anion scavenging activity	Nitric oxide scavenging activity	Antihaemolytic activity	Metal chelating activity
Extract	73.29±0.81	89.03±1.07	84.25±1.18	79.09± 0.75	64.12±0.11
Ascorbic acid	67.47±0.09	92.22±0.40	91.66±1.01	-	-
BHT	61.31±1.25	-	-	-	-
BHA	54.11±1.95	73.36±1.31	-	82.12± 0.13	-
α-Tocopherol	67.26±0.22	78.16±1.14	-	74.43± 0.39	-

Data are expressed as means ± standard deviations (n = 3) on dry weight basis

DISCUSSION

Numbers of phytoconstituents are present in nature, out of them poly phenols is one of the major class, which are natural antioxidants (Chew *et al.,* 2009). Our findings showed that MeOH extract of *P. pterocarpum* pods was found to have a high phenolic contents. The redox property of pod extract is measured by DPPH, widely used method to evaluate the free radical scavenging ability in various plant species in short period (Ebrahimzadeh *et al.,* 2009a). It showed an excellent scavenging property which was more than that of standards including ascorbic acid, BHT, BHA and α-Tocopherol, given in fig. 1.

Superoxide anion radical is another reactive oxygen species among the free radicals (Garrat, 1964) which indirectly initiates lipid peroxidation because superoxide anion acts as a precursor of singlet oxygen and hydroxyl radical (Ebrahimzadeh *et al.,* 2009b). Vitamin C is involved in the detoxification of free radicals and more effective in lipid peroxidation (Estuo *et al.,* 1995). In this connection, the test plant extract was suggested to have an effective scavenger effect on superoxide radical as well.

Moreover to reactive oxygen species, Nitric oxide is also concerned in inflammation, cancer and other pathologies (Nabavi *et al.,* 2008). The pod extract displayed considerable Nitric oxide scavenging activity too. The extract showed to decrease nitrite formation by competing oxygen to counter with nitric oxide directly and also to reduce its synthesis (Marcocci *et al.,* 1994), which may prevent the week effects of unwanted NO generation in the human body.

Chelation property may give defence against oxidative damage and iron-overload (Lai *et al.,* 2001) because metal chelators as iron chelators mobilize tissue iron by forming soluble and stable complexes and reduces iron-related difficulties in humans and excreted out from the body. Hence they improve worth of life and overall endurance against various live threatening diseases such as thalassemia major and Alzheimer's disease (Ebrahimzadeh *et al.,* 2008, 2009c). The pods extract

exhibited good iron chelating activity and provides a strategy to avoid free-radical generation and iron-overload by chelation of metal ion (Robak *et al.,* 1988).

Haemolysis has a long history of measuring damage caused by free radicals and its inhibition by antioxidants (Chew *et al.,* 2009). Erythrocytes are considered as prime targets of free radicals. Non toxic effect of the sample extract suggested that the extract may be used for the treatment of various disorders (Chaudhuri *et al.,* 2007; Magalhaes *et al.,* 2009; Ebrahimzadeh *et al.,* 2010; 2009d).

The preliminary antioxidant findings of pod extract may provide the base for selection and isolation of new plants having bioactive compound like phenols which has strong antioxidant activity. There are number of reports that proved that phenols have scavenging ability due to the presence of hydroxyl group. It was also reported they are effective hydrogen donors and the position and degree of hydroxylation of phenolic compounds especially in the B-ring play a major role make them excellent antioxidants (Fukumoto and Mazza, 2000). In the present study, 80% methanolic extract of *Peltophorum pterocarpum* pods have notable antioxidant, free radical scavenging and anti-haemolytic activities. These studies showed that this plant extract could be a new potential source of natural antioxidants which may contributing in the prevention of various degenerative diseases which are very common nowadays.

REFERENCES

Anonymous (1966). The Wealth of India, CSIR New Delhi, 7: 291.

Barry H (1991). Antioxidant affects a basis of drug selection. *Drugs,* **42**(2): 569-605.

Chaudhuri S, Banerjee A, Basu K, Sengupta B and Sengupta PK (2007). Interaction of flavonoids with red blood cell membrane lipids and proteins: Antioxidant and antihemolytic effects. *Int. J. Biol. Macromol.,* **41**: 42-48.

Chew YL, Goh JK and Lim YY (2009). Assessment of *in vitro* antioxidant capacity and polyphenolic composition of selected medicinal herbs from *Leguminosae* family in Peninsular Malaysia. *Food Chem.,* **119**: 373-378.

Derjani C and Barkha S (2011). Antimicrobial, anti oxidative and anti hemolytic activity of *Piper betal* leaf extracts. *Int. J. Pharm. Pharm. Sci.,* **3**: 192-199.

Duraipandayan V, Ayyanar M and Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC complement. Alten. Med.*, **6**(35): 1472-6882.

Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR (2008). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr. J. Biotechol.,* **7**(18): 3188-3192.

Ebrahimzadeh MA, Ehsanifar S and Eslami B (2009a). *Sambucusebuluselburensis* fruits: A good source for antioxidants. *Pharmacog. Mag.*, **4**(19): 213-218.

Ebrahimzadeh MA, Nabavi SF, Eslami B, Nabavi SM (2009)b. Antioxidant and antihemolytic potentials of *Physospermum cornobiense* (L.) DC. *Pharmacol. Online.,* **3**: 394-403.

Ebrahimzadeh MA, Nabavi SM and Nabavi SF (2009c). Correlation between the *in vitro* iron chelating activity and polyphenol and flavonoid contents of some medicinal plants. *Pak. J. Biol. Sci.,* 12(12): 934-938.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM (2009)d. Essential oil composition and antioxidant activity of *Pterocarya fraxinifolia*. *Pak. J. Biol. Sci*., **12**(13): 957-963.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM and Eslami B (2010). Antihemolytic and antioxidant activities of *Allium paradoxum. Centr. Eur. J. Biol.,* **5**(3): 338-345.

Estuo N, Norkio N, Hideyasu T and Naohiro G (1995). Interaction among vitamin C, vitamin E and β-carotene. *Am. J. Clin. Nutr.,* **62**: 1322-1326.

Fukumoto LR, Mazza G (2000). Assessing antioxidant and prooxidant activity of phenolic compounds. *J. Agri. Food Chem.,* **48**(8): 3597-604.

Garrat DC (1964). The quantitative analysis of drugs. 3rd edition. Chapman and Hall, Japan, pp.456-458.

Govindarajan R, Rastogi S, Vijayakumar M (2003). Studies on antioxidant activities of *Desmodium gangeticum*. *Bio. Pharm. Bull.,* 26: 1424-1427.

Ilhami GI, Haci AA and Mehmet C (2005). Determination of in vitro antioxidant and radical scavenging activities of Propofol. *Chem. Pharm. Bull.*, **53**: 281-285.

Jagessar RC, Mohamed A and Gomes G (2007). An evaluation of the antibacterial and antifungal activity of leaf extracts of *Luffa operculata*, vs *Peltophorum Pterocarpum*, against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. *Nat. and Sci.,* **5**(4): 81-93.

Jain SC, Pancholi B and Jain R (2011). *Peltophorum pterocarpum* (D.C.) ex K. Heyne Flowers. Antimicrobial and antioxidant efficacies. *Res. J. Med. Plant.,* **5**(3): 274-280.

Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K and Kujala TS (1999). Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food. Chem.,* **47**: 3954-3962.

Lai HY, Lim YY and Kim KH (2010). *Blechnum Orientale* Linn - a fern with potential as antioxidant, anticancer and antibacterial agent. *BMC Complement. Altern. Med.,* **15**: 1472-6882.

Ling LT, Radhakrisnan AK, Subramaniam T (2010). Assessment of antioxidant capacity and cytotoxicity of selected Malaysian plants. *Molecules,* **15**(3): 2139-2151.

Magalhaes AS, Silva BM, Pereira JA, Andrade PB, Valentao P and Carvalho M (2009). Protective effect of quince (*Cydoniaoblonga Miller*) fruit against oxidative hemolysis of human erythrocytes. *Food Chem. Toxicol.,* **47**: 1372-1377.

Manaharan T, Teng LL, Appleton D, Ming CH, Musilamani T and Palanisamy UD (2011). Antioxidant and anti glycemic potential of *Peltophorum pterocarpum* plant parts. *Food Chem.,* **129**: 1355-1361.

Marcocci L, Packer L, Droy-Lefai MT, Sekaki A and Gardes-Albert M (1994). Antioxidant action of *Ginkgo biloba* extracts EGb 761. *Methods Enzymol*., **234**: 462-475.

Nabavi SM, Ebrahimzadeh MA, Nabavi SF, HamidiniaA, Bekhradnia AR (2008). Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey*. *Pharmacologyonline,* **2**: 560-567.

Naim M, Gestener B, Bondi A and Birk Y (1976). Antioxidant and anti haemolytic activities of soyabean isoflavones. *J. Agric. Food Chem.,* **24**: 1174-1177.

Robak J and Gryglewski RJ (1988). Flavonoids are scavengers of superoxide anion. *Biochem. Pharmacol.*, **37**: 83-8.

Sabate J (2003). The contribution of vegetarian diets to health and disease: A paradigm shift. *Am. J. Clinic. Nutrit.,* **78**: 502-507.

Sethuraman MG, Sulochana N and Kameswaran L (1984). Anti-inflammatory and antibacterial activity of *Peltophorum pterocarpum* flowers. *Fitoterapi.*, **55**(3): 177-179.

Sharma V, Paliwal R and Sharma S (2011). Phytochemical analysis and evaluation of antioxidant activities of hydro-ethanolic extract of *Moringa oleifera* Lam pods. *J. Pharm. Res*., **4**: 554-557.

Valdlapudi V and Naidu K C (2010). *In vitro* antimicrobial screening of selected Indian medicinal plants. *Int. J. App. Biol. Pharm. Tech.,* **1**: 302-307.

Yafang S, Gan Z and Jinsong B (2011). Total phenolic content and antioxidant capacity of rice grains with extremely small size. *Afr. J. Agricult. Res.,* **6**(10): 2289-2293.

Yen GC, Duh PD and Tsai CL (1993). Relationship between antioxidant activity and maturity of peanut hulls. *J. Agric. Food. Chem.,* **41**: 67-70.