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

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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.

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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 vanilane 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 (Duraiapadayan et al., 2006; Valdilapudi et al., 2010). The leaf and bark of this plant reported to contain phenolic compounds that showed antibacterial, antioxidant and hypoglycemnic activity (Jagessar et al., 2011; 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 antihaemolymic 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, garbeled 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-picyrylhydrazyl radical (DPPH’), 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).

**DPH 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:

\[
\text{Inhibition (\%) } = \frac{A_o - A_i}{A_o} \times 100
\]

where \( A_o \) = absorbance of the control and \( A_i \) = absorbance of the extract. Scavenging activity was compared with standards like ascorbic acid, \( \alpha \)-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:
Inhibition (%) = \( \frac{A_0 - A_1}{A_0} \times 100 \)

where \( A_0 \) = absorbance of the control and \( A_1 \) = absorbance of the extract (Ilhami et al., 2005). BHA and \( \alpha \)-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 \( \mu \)g/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-naphthylethlenediamine dihydrochloride). During reaction, diazotization of nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethlenediamine 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 \( \text{Fe}^{2+} \text{–Ferrozine} \) complex was measured spectrophotometrically 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 \( \mu \)g/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 \( \text{H}_2\text{O}_2 \) solution of appropriate concentration in saline buffer was added to mixture to induce oxidative degradation in the lipoprotein membrane. The concentration of \( \text{H}_2\text{O}_2 \) in the reaction mixture was adjusted to bring approximately 90% haemolysis in blood cells. The reaction mixture was then centrifuged at 800 \( \times \) 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 \( \alpha \)-tocopherol and BHA.

**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>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE/g)</th>
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<tr>
<td><em>Peltophorum pterocarpum</em> extract</td>
<td>439.21 ± 0.17</td>
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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 \( \alpha \)-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 \( \alpha \)-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., 82.12±0.13% compared with ascorbic acid as standard as shown in table 2.
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 \textit{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 \(\alpha\)-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 antihaemolytic 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


