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Invitro Antioxidant Activity of *Delonix elata* L

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ABSTRACT

Antioxidant activity of ethanolic extract of *Delonix elata* L.was studied for its free radical scavenging property on different in vitro models e.g 1, 1-dipheny 1-2-picryl hydrazine (DPPH), hydrogen peroxide, total antioxidant capacity and peroxy radical model. The invitro lipid peroxidation (LPO) was inhibited to a good extent by the ethanolic leaf extract of *Delonix elata* L. The extract showed good dose – dependent free radical scavenging property and antioxidant property when compared to standards. In all the models, the results obtained in the present study indicate that the leaves of *D.elata* L. are a potential source of natural antioxidant.

INTRODUCTION

Oxygen is essential for the survival of all on this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals [1,2] like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. All the radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cells of human body rendering each cell to face about 10000 oxidative hits per second [3]. When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates [4-6] and this leads to a number of physiological disorders. Free radicals are involved in the development of degenerative diseases [6]. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardio-vascular disorders, neurological disorders, and in the process of aging [7]. Many plants often contain substained amounts of antioxidants including vitamin C and Vitamin E, catenoids, flavonoids and tannins etc., and thus can be utilized to scavenge the excess free radicals from human body [8].

Delonix elata L, is a deciduous tree about 2.5-15 m tall, found in India and Srilanka. The plant is traditionally used for the treatement of rheumatism and flatulence. It's stem bark is considered a good febrifuge and is much appreciated as an antiperiodic. The leaf extract are anti-inflammatory, a root decoction is drunk for abdominal pains. A psychosomatic medicinal use relating to scorpion bite treatment is reported from India [9]. The objective of the present study was to investigate the invirtro antioxidant activity of different concentrations of the ethanolic leaf extract of *D.elata* L.

MATERIALS AND METHODS

The chemicals used are 1,1.-dipheny1-2-picryl hydrazyl (DPPH), ascorbic acid, α-tocopherol, hydrogen peroxide solution, linoleic acid emulsion, ammonium thiocyanate, ferrous chloride, hydrocholoric acid, sulfuric acid, sodium phosphate, ammonium molybdate and were of analytical grade.

Plant material – The leaves of *D.elata* L.was collected during May 2008 from Nagapattinam District, Tamilnadu, India. The leaves were dried under shade and then powdered in a mechanical grinder. The powdered material was extracted successively with ethanol(70%) using soxhlet appartus. The ethanolic extract was concentrated in vacuo and kept in a vaccum dessicator for complete removal of solvent. The yield was 9.1% w/w with respect to dried powder. The ethanol extract of *Delonix elata* L. (EEDE) was used for the antioxidant studies.

Invitro antioxidant study

EEDE was tested for its free radical scavenging property using different invitro models. All experiments were performed six times and the results were averaged.

DPPH radical scavenging assay

The radical scavenging activity of the fractions was measured in vitro by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay [10]. About 0.3mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations (20-100μg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and the IC_{50} value of the fractions was compared with that o ascorbic acid, which was used as the standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2mM/L) was prepared with standard phosphate buffer (PH 7.4). Different concentration of the fraction (20-100µg/ml) in distilled water was added to 0.6ml of hydrogen peroxide solution. Absorbance was determined at 230nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and the IC_{50} values were compared, α-tocopherol [11] with the standard.

Total antioxidant capacity

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using, α-tocopherol as the standard [12]. An aliquot of 0.1ml of the fractions (20-100µg/ml) solution was combined with 1.0 ml of reagent (0.6M sulfuric acid, 28m M sodium phosphate and 4mM ammonium molybdate). The tubes were capped and inclubated in a boiling water bath at 95˚C for 90 min. After the samples had cooled to room temperature. The absorbance was measured at 695 nm against the blank using an UV spectrophotometer. The blank solution contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. The total antioxidant capacity was expressed as mg equivalents of α-tocopherol by using the standard tocopherol graph.

Peroxy radical scavenging activity

The peroxy radical scavenging activity was determined by thiocianate method using α-tocopherol (20-100 µg /ml) as standard [13]. Increasing concentration of the fractions (20-100 µg /ml) in 0.5ml of distilled water and mixed with 2.5 ml 0.02M linoleic acid emulsion (in 0.04M phosphate buffer PH 7) in a test tube and incubated in darkness at 37˚C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocianate solution and 0.1ml of 20mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentage scavenging activity was calculated and the IC_{50} values of the fractions were compared with the standard α-tocopherol.

Calculation of 50% Inhibitory Concentration (IC_{50}).

The concentration of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhabition (1%) was calculated using the formula

$$1\% = \frac{(Ac - As)}{Ac} \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Statistical analysis

All experiments were performed in six times (n=6) and results were expressed as mean ± SEM.

RESULTS

The ethanolic extract of *Delonix elata* L. was subjected to screening for their possible antioxidant activity. Four complementary test systems, namely DPPH radical scavenging, hydrogen peroxide scavenging, total antioxidant capacity, paroxy radical scavenging were used for the analysis.

The free radical scavenging activity of *Delonix elata* L. by DPPH method was done (Table-1). The extract shows the inhibitory percentage of about 55, 64, 67, 78, 88 (ug/ml) at various concentrations of 20, 40, 60, 80, 100 (µg /ml) respectively. All the fractions of the *Delonix elata* L. demonstrated H-donor activity. These activities are less than that of ascorbic acid 58.4, 69.7, 77.3, 86.7, 95.3 (µg /ml) respectively. The inhibitory percentage increases with increase in concentration of the substrate.

The hydrogen peroxide radical scavenging activity of *Delonix elata* L indicated that the ethanolic extracts of *Delonix elata* L. exhibits inhibitory percentage of about 47.2, 59.9, 65.9, 75.3, 85.2 µg /ml at various concentrations of 20, 40, 60, 80,100 µg /ml respectively(Table-2). These activities are greater than that of α-tocopherol (41.3, 50.5, 61.9, 71.2, 77.5 µg /ml) respectively.

The total antioxidant capacity of sethanolic extract of *Delonix elata* L was found by phosphomolybdate method(Table-3). The total antioxidant capacity of ethanolic extracts of *Delonix elata* L. in inhibitory percentage of about 16.22, 31.04, 49.40, 66.02, 81.42 (µg/ml) at various concentrations 20, 40, 60, 80, 100 µg/ml respectively when compared to α-tocopherol.

Table 1: Free radical scavenging activity of ethanolic extract of *Delonix elata* L. (DPPH Assay).

Concentration of the extract and standard (ascorbic acid) (µg/ml)	Inhibitory percentage (µg/ml)	
	Extract	Standard
20	55 ±1.1	58.4 ± 1.21
40	64 ± 0.5	69.7 ± 1.15
60	67 ± 1.1	77.3 ± 1.0
80	78 ± 2.0	86.7 ± 1.09
100	88 ± 0.8	95.3 ± 0.95

Values are expressed as mean standard deviation

Table 2: Hydrogen peroxide radical scavenging activity of ethanolic extract of *Delonix elata* L.

Concentration of the extract and standard (α – tocopherol) (µg/ml)	Inhibitory percentage (µg/ml)	
	Extract	Standard
20	47.2 ± 1.5	41.3±1.0
40	59.5 ± 0.75	50.5 ± 1.3
60	65.9 ±1.96	61.9 ± 0.7
80	75.3 ± 2.1	71.2 ± 1.1
100	85.2± 0.95	77.5 ± 1.8

Values are expressed as mean standard deviation

The amount of formed peroxides measured by thiocyanate method was compared with α-tocopherol(Table-4). The ethanolic extract of *Delonix elata* L. Shows increased values of inhibitory percentage in various concentrations at various intervals (15, 30, 45) when compared to α-tocopherol.

DISCUSSION

DPPH assay is one of the widely used for screening antioxidant activity of plant extracts DPPH is a stable, nitrogen-centered free radical, which produces violet colour in ethanol solution. It was reduced to a yellow coloured product, diphneyl picrylhydrazine, with the addition of the fractions in a concentration –dependent manner. All the fractions showed significantly higher inhibitory percentage.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals, which is very toxic to the cell [14]. Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the fractions. All the fractions of *Delonix elata* L. Scavenged hydrogen peroxide, which may attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide there by neutralizing it into water.

Table 3: Total antioxidant activity of ethanolic extract of *Delonix elata* L.by phosphomolybdate method.

Concentration of the extract and standard (α – tocopherol) (µg/ml)	Inhibitory percentage (µg/ml)	
	Extract	Standard
20	16.22 ± 0.11	12.10 ± 0.07
40	31.04 ± 0.02	27.12 ± 0.02
60	49.40 ± 0.23	44.20 ± 0.20
80	66.02 ± 0.14	62.12 ± 0.10
100	81.42 ± 0.04	77.14 ± 0.02

Table 4: Determination of peroxy radical scavenging activity of ethanolic extract of *Delonix elata* L.

Concentration of the sample and standard (α-tocopherol) (µg/ml)	Inhibitory percentage (µg/ml)					
	15 (min)		30 (min)		45 (min)	
	Extract	Standard	Extract	Standard	Extract	Standard
20	11.5 ± 0.03	12.7± 0.05	22. ± 1.2	24 ± 1.3	32.2 ± 1.2	34.1 ± 1.3
40	17.3 ± 0.1	19.5 ± 0.2	28 ± 0.9	30 ± 0.9	42.1 ± 1.1	44.1 ± 1.2
60	22.3±1.2	24.7 ± 1.5	35.5 ± 1.35	37.5 ± 1.4	50.3 ± 1.35	53.4 ± 1.4
80	29.3 ± 1.9	32.2 ±1.9	42.1± 1.1	44.3 ± 1.2	56.1 ± 2.5	58.2 ± 2.7
100	37.5 ± 0.5	40.5 ± 0.7	49.3 ± 1.4	49.3 ± 1.7	61.7 ± 2.3	63.9 ± 2.3

Values are expressed as mean standard deviation

In the presence of fraction, the molybdenum(VI) is reduced to molybdenum(V) and forms a green colour complex which shows maximum absorbance at 695 nm [15]. Increase in percentage inhibition shows that the ethanolic extracts of *Delonix elata* L. has significant antioxidant activity.

The fractions were circulated with linoleic emulsion in dark at 37°C and the amount of peroxides was determined spectrophotometrically by measuring the absorbance of 500 nm [16]. The absorbance decrease with increasing concentration of the fractions indicated the antioxidant activity of *Delonix elata* L. while it might be due to the inactivation of free radicals.

A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom [17,18]. Based on the results obtained, it may be concluded that all the fractions of the ethanolic extract of the leaves of *D.elata* L. showed strong antioxidant activity and free radical scavenging activity. Further studies are needed to evaluate the in vivo potential of the fractions in various animal models and the isolation and identification of the antioxidant principles in the leaves of *Delonix elata* L. are being carried out.

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