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ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *DELONIX ELATA* (FLOWERS)

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ABSTRACT

Delonix elata L. (Ceasalpinaceae family), is extensively used by the traditional medical practitioners of Karnataka, India, to cure jaundice, bronchial and rheumatic diseases. The present study was to screen the *in vitro* antioxidant and anti-inflammatory behaviors of the sample isolated from the ethyl acetate fraction of flowers of *Delonix elata.* Anti-inflammatory activity of the sample was determined by HRBC membrane stabilization and Albumin denaturation methods. Anti-oxidant activity of the sample was determined by DPPH assay and ABTS method. *In vitro* antioxidant and anti-inflammatory activity revealed that the sample isolated from the ethyl acetate fraction showed more potent anti-oxidant and anti-inflammatory activities. Further studies will be needed in future in order to determine which one or more of its active constituents have the major antioxidant and anti-inflammatory effects.

KEYWORDS: *Delonix elata*, Antioxidant activity, Anti-inflammatory activity, HRBC method, Albumin denaturation, DPPH, ABTS assay.

INTRODUCTION

Antioxidants are an inhibitor of the course of oxidation, even at moderately small concentration and thus have diverse physiological function in the body. Antioxidants may be man-made or natural. man-made antioxidants such as BHT and BHA have recently been reported to be hazardous for human health. Thus, the search for effective, non-toxic natural compound with anti oxidative activity has been intensified in modern years.[1] *Delonix elata* is a hermaphroditic, deciduous tree. Flowers in the hot time of year or for the duration of the early rains, in east Africa this is usually around December and August-March in India. Fruit ripening is between May and July**.** *Delonix elata* is called Gul mohar or Gold mohur, generally well-known as vadhamudakki in Tamil belongs to Ceasalpinaceae.[2],[3] A prominently ornamental average – sized tree, planted in avenues and gardens in all warmer and damper parts of India. It has a spreading crown of feathery foliage and bears flowers early in the hot season as the foliage falls and the branches are nearly bare. The leaves and barks of the plant are widely used by Siddha and Ayurveda practitioners for treating a number of conditions. *Delonix elata* used as anti – inflammatory, rheumatism,[4] anti-microbial and have antioxidant.[5,6,7] The medical signs that inflammation evoke are heat, redness, bulge and loss of function. Anti-inflammatory agent is a drug that inhibits any facet of inflammation of an experimentally induced nature or as a part of clinical syndrome.[8]

MATERIALS AND METHODS

Collection of Flowers

Fresh flowers of *Delonix elata* were collected from Karaikudi, Sivagangai (Dt), Tamil Nadu, India, during the month of April and identified by Dr.S.John Britto, Director, The rapinat Herbarium and Centre for Molecular Systematics (Authentication No. AR004 dated: 05/04/2017). St.Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation

Fresh flowers (3 kgs) of *Delonix elata* collected were extracted with 90% ethanol (5x500ml). The combined alcoholic extract was concentrated in vacuo and the aqueous extract was successively fractionated with petroleum ether (60-80^{0}C) (6x250ml), Peroxide free diethyl ether (4x250ml) and ethyl acetate (8x250ml). Petroleum ether fraction and diethyl ether fraction did

not yield any isolable material. Ethyl acetate fraction on concentration yielded a dry powder which was dissolved in DMSO to get various concentrations and were used for further study.

In vitro antioxidant activity

DPPH Assay Method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm.[9]

Reagents

A. 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100µM)

22mg of DPPH was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100µM DPPH solution.

B. Preparation of test solutions

21 mg of the solid obtained from ethyl acetate fraction was dissolved in distilled DMSO to obtain a solution of 21mg/ml concentration. This solution was serially diluted to obtain lower concentrations.

C. Preparation of standard solutions

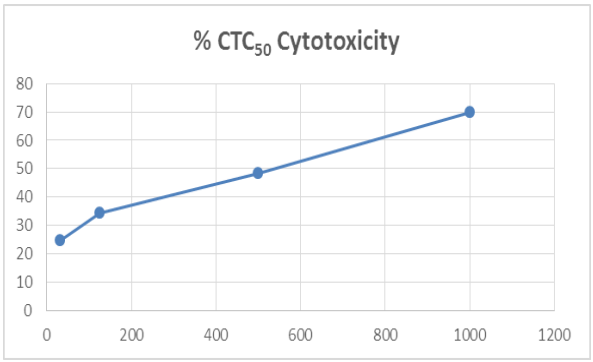
10mg of ascorbic acid was weighed and dissolved in 1ml of Dimethyl sulfoxide (DMSO) to get 10mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

D. Procedure

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the micro titre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 µg/ml. The plates were incubated at 37^0C for 30 min and the absorbance of each solution was measured at 490 nm, using a micro plate reader.

Table No. 1: Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata by DPPH assay.

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity (µg/ml)	IC_{50} (µg/ml)
1	1000	69.86	542.2
2	500	48.41	
3	125	34.24	
4	31.25	24.65	



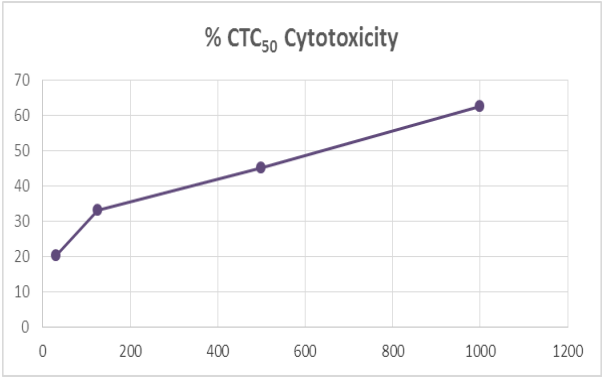
Graph No.1: Graphical representation of anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata by DPPH assay.

ABTS radical scavenging activity

ABTS radical scavenging activity was performed as described by Re *et al.* (1999) with a slight modification. 7.0 mM ABTS in 14.7 mM ammonium peroxo-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution (in ethanol) (20.0 µl) were added to 980.0 µL of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µL of ethanol processed as described above was served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

Table No. 2: Anti-oxidant activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata by ABTS assay.

S. No	Concentration (µg/ml)	% CTC_{50} Cytotoxicity(µg/ml)	IC_{50} (µg/ml)
1	1000	62.48	659.5
2	500	45.19	
3	125	32.96	
4	31.25	20.15	



Graph No.2: Graphical representation of radical scavenging activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata by ABTS assay.

Anti- inflammatory activity

The human red blood cell (HRBC) membrane stabilization method

The method as prescribed (Gopalkrishnan *et al*., 2009; Sakat *et al*., 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of test drug were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37^0C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100Jg/ml) was used as reference standard and a control was prepared by omitting the test drug. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection was calculated.[10,11]

Percentage of Protection (%) =

(100-OD of drug treated sample/OD of Control) × 100

Albumin denaturation method

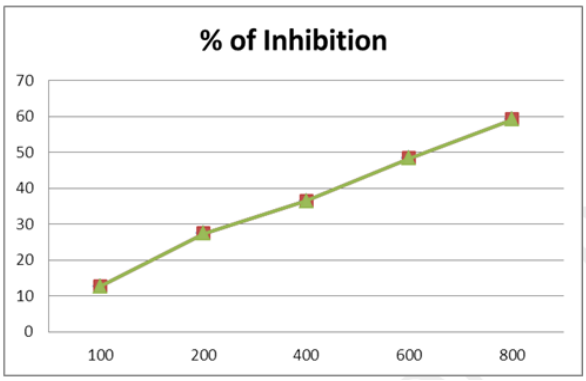
The method as prescribed (Sakat *et al*., 2010) was followed with some modifications. The reaction mixture was consisting of test sample and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The mixtures were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows,[12,13]

Percentage of inhibition (%) =

(OD of Control- OD of Sample/ OD of Control) X 100

Table 3: The human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata.

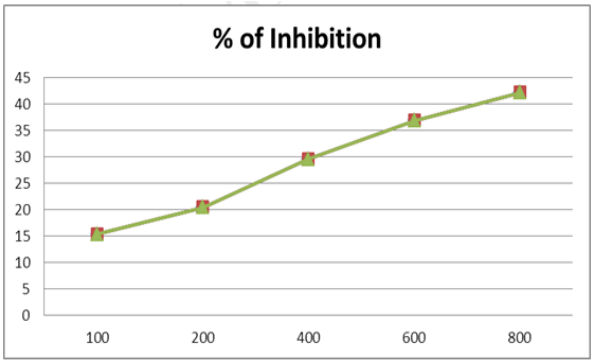
S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M(S-I)
1	100	12.69 ± 0.48
2	200	27.36 ± 0.74
3	400	36.41 ± 0.84
4	600	48.39 ± 1.85
5	800	59.15 ± 1.69



Graph 3: Graphical representation of human red blood cell (HRBC) membrane Stabilization activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata.

Table 4: The Inhibition of Albumin Denaturation activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata.

S. No	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S.E.M(S-I)
1	100	15.39 ± 0.89
2	200	20.48 ± 0.78
3	400	29.61 ± 0.47
4	600	36.89 ± 1.90
5	800	42.18 ± 1.75



Graph 4: Graphical representation of Inhibition of Albumin Denaturation activity of the compound isolated from the ethyl acetate fraction of flowers of Delonix elata.

RESULTS AND DISCUSSION

Anti-oxidant activity

The compound isolated from the ethyl acetate fractions of *Delonix elata* flowers exhibited significant anti-oxidant activity which is evident from the data presented in Table-1. The result showed the percentage of cytotoxicity for 1000 µg/ml as 69.86 %, for 500 µg/ml as 48.41 %, for 125 µg/ml 34.24 %, and for 31.25 µg/ml 24.65 %. When compared with ABTS assay activity, it is evident from the data presented in Table II that the sample possesses ABTS assay activity. The result showed the percentage of cytotoxicity for 1000 µg/ml as

62.48 %, for 500 µg/ml as 45.19 %, for 125 µg/ml as 32.96 %, and for 31.25 µg/ml as 20.15 %.

Anti-inflammatory activity

The compound isolated from the ethyl acetate fractions of *Delonix elata* flowers exhibited significant anti-inflammatory activity of the human red blood cell (HRBC) membrane stabilization and the results are presented in Table III. The result showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 12.69 ± 0.48 % , for 200 µg/ml as 27.36 ± 0.74 % , for 400 µg/ml as 36.41 ± 0.84 % , for 600 µg/ml as 48.39 ± 1.85 % , and for 800 µg/ml as 59.15 ± 1.69 %. The inhibition of Albumin denaturation activity exhibited by the compound are given in Table IV. The results showed the percentage of inhibition in membrane stabilization for 100 µg/ml as 15.39 ± 0.89 %, for 200 µg/ml 20.48 ± 0.78 %, for 400 µg/ml as 29.61 ± 0.47 %, for 600 µg/ml as 36.89±1.90 %, and for 800 µg/ml as 42.18 ±1.75%. The anti-inflammatory effect of the compound isolated from ethyl acetate fraction (test sample) of *Delonix elata* may be due to presence of active constituent flavonoids. The results strongly suggest anti-inflammatory effects and anti-oxidant effects by percentage of inhibitions, which are explained in the Table 1,2,3,4.

CONCLUSION

The present study has confirmed that both DPPH assay and ABTS have showed a highest potential antioxidant activity and also the human red blood cell (HRBC) membrane stabilization. It could be concluded that the compound isolated from the ethyl acetate fraction of flowers of *Delonix elata* is of phytopharmaceutical importance.

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