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IN-VITRO ANTIOXIDANT PROPERTIES OF *DELONIX ELATA* WHOLE PLANT EXTRACTS

B. S. Sharvana Bhava [1], K. Umasankar [1] and A. Kottai Muthu [*2]

Department of Pharmacology [1], Vaagdevi College of Pharmacy, Warangal - 506001, Telangana, India.
Department of Pharmacy [2], Faculty of Engineering & Technology, Annamalai University, Annamalai Nagar, Chidambaram - 608002, Tamil Nadu, India.

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Correspondence to Author:
Dr. A. Kottai Muthu

Assistant Professor,
Department of Pharmacy,
Faculty of Engineering & Technology,
Annamalai University, Annamalai
Nagar, Chidambaram - 608002,
Tamil Nadu, India.

E-mail: arthik03@yahoo.com

ABSTRACT: *Delonix elata* (*D. elata*) whole plant was chosen to evaluate *in-vitro* anti-oxidant activities. The total polyphenols and flavonoids were determined after the extraction. DPPH (2, 2'-diphenyl-1-picrylhydrazy), H_2O_2 (Hydrogen peroxide), NO (Nitric oxide) and TBARS (Thiobarbituric acid) methods were used to evaluate antioxidant activities of *D. elata* whole plant. The results showed that the highest levels of polyphenols were recorded in DEM (15.32 ± 0.32 mg of GA/g of extract). Total flavonoid contents of extracts/fractions showed that DEM (15.57 ± 0.69 mg of Qu/g of extract) contains the highest level. The DEM extract shows more inhibition of DPPH (92.56), NO (143.88), H_2O_2 (182.28) and TBARS (121.94 µg/ml), respectively when compared with other extracts. These findings suggest that the extracts obtained from *D. elata* stem have active substances contributing to the increase in natural antioxidant potential. It can be concluded from the findings that *D. elata* extracts have potent antioxidant potential.

INTRODUCTION: The substance even in lower concentrations which oppose or delay oxidation of a substrate is called an anti-oxidant. Normal cellular metabolisms form reactive oxygen species (ROS) and reactive nitrogen species (RNS), which cause many diseases due to oxidative stress. Thus research on various anti-oxidants with great anti-oxidant potential is worth of importance [1, 2]. Two mechanisms *viz.*, A) Obstructing the free radical production B) protecting the cell membrane by free radical scavengers such as catalase (CAT), glutathione peroxidase (GPx) and super oxide dismutase (SOD) are postulated for antioxidant activity [3].

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The major source of antioxidants till today is the medicinal plants [4]. *Delonix elata* belongs to the family Caesalpiniaceae, and it is widely found in south India. Presence of glycosides, flavonoids, phenols, saponins, tannins, and steroids were found in the preliminary phytochemical screening of the extract of the aerial parts. The motto of this work was to substantiate the *in-vitro* antioxidant activities of aerial parts of *Delonix elata* petroleum ether, ethyl acetate, and methanolic extracts respectively.

MATERIALS AND METHODS:

Reagents and Standards: Petroleum ether, ethyl acetate, methanol, gallic acid, quercetin, aluminum-chloride, 1, 1-diphenyl-2-picrylhydrazyl (a a-diphenyl-b-picrylhydrazyl; DPPH), H_2O_2, griess reagent, trichloroacetic acid, thiobarbituric acid.

Plant Materials: The aerial parts of *D. elata* were collected in parts of Kerala. The collected whole plant was authenticated by Dr. V. Chelladurai,

Research Officer- Botany (Scientist -C) and Authentication no. is BSI/SRC/5/23/2017/Tech/727 Central Council for Research in Ayurveda & Siddha, AYUSH, Government of India.

Preparation of Extracts: The collected material was washed with distilled water and dried at room temperature to constant weights and ground to powder. The powder (UNIT Quantity) was sealed in a muslin cloth and Soxhlated with a pet. ether, ethyl acetate, and methanol as solvents respectively according to the increasing order of polarity. Each extract was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator.

Determination of Total Phenolic Content (TPC): The three extracts were evaluated for total phenolic content with Folin-Ciocalteu method [5]. Gallic acid calibration curve was used for estimation of the phenolic content of three extracts. Sample determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

Determination of Total Flavonoid Content (TFC): Total flavonoid content in the extracts was determined by aluminum-chloride colorimetric assay [6]. The determination of total flavonoid content in the three extracts and standard were performed thrice. The result was determined from the calibration curve and expressed as milligram of quercetin equivalent (QE) per gram of extracts [7].

***In-vitro* Antioxidant Activity:**

DPPH Radical Scavenging Activity: The antioxidant potential of the three extracts was measured based on the scavenging of DPPH (1, 1-diphenyl-2-picrylhydrazyl (an a-diphenyl-b-picrylhydrazyl)).

According to Manzocco *et al.*, the sample extract (0.2 mL) is diluted with methanol, and 2 mL of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm using UV-Visible spectrophotometer. The percentage inhibition of DPPH radical can be extracted from the equation as follows:

$$\% \text{ inhibition of DPPH radical} = \{(Abr - Aar)/ Abr\} \times 100$$

Where Abr is the absorbance before reaction and Aar is the absorbance after the reaction has taken place [8].

Hydrogen Peroxide Scavenging (H_2O_2) Assay: The ability of the extract to scavenge hydrogen peroxide is estimated by following the method of Ruch *et al*. [9]

Sample extracts are added prepared hydrogen peroxide solution, and absorbance is determined after 10 min at 230 nm against a blank solution (Phosphate buffer without hydrogen peroxide). The percentage scavenged by the sample can be determined by:

$$\% \text{ scavenged } (H_2O_2) = \{(Ai - At)/ Ai\} \times 100$$

Where Ai is the absorbance of the control and At is the absorbance of the test.

Nitric Oxide Scavenging Activity: 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) is mixed with 0.5 mL of sample. After 150 min of incubation at 25 °C, 0.5 ml of the incubated solution is withdrawn and mixed with 0.5 mL of Griess reagent [(1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthyl ethylenediamine dichloride (0.1% w/v)]. This is then incubated at room temperature for 30 min, and its absorbance was measured at 546 nm. The percentage inhibition of NO radical can be obtained by:

$$\% \text{ inhibition of NO radical} = \{(A0 - A1)/ A0\} \times 100$$

Where, A0 is the absorbance before reaction, and A1 is the absorbance after the reaction has taken place with Griess reagent [10].

TBARS Assay: The final sample concentration of 0.02% w/v, as described by Ottolenghi *et al.*, was used in this method. To 1 ml of the sample solution, 2 mL of 20% trichloroacetic acid and 2 mL of 0.67% of thiobarbituric acid were added and kept in a boiling water bath for 10 min. The mixture was cooled and then centrifuged for 20 min at 3000 rpm. The supernatant absorbance was measured at 552 nm and recorded after it has reached its maximum [10].

Statistical Analysis: The results were expressed as Mean ± SD. The data were analyzed using one-way analysis of variance (ANOVA), and the differences between samples were determined by Dunnett's test, $P<0.05$ were regarded as significant.

RESULTS AND DISCUSSION:

Total Phenolic and Flavonoid Content: The total phenolic and flavonoid content of the petroleum ether, ethyl acetate and methanolic extracts of *Delonix elata* (DE) were done by Folin-Ciocalteu colorimetric and $AlCl_3$ methods, respectively. The total phenolic contents were expressed as mg gallic acid equivalent per gram of dry extract. In **Table 1**, phenolic content in the examined whole plant extracts of DE ranges from 0.32 to 15.32. The DEM has very high values of total phenolic contents (15.32) when compared with remaining extracts like DEP (0.32) and DEE (5.72). In the analysis of results for the concentrations of total phenolic compounds extracts, it is noticed that the highest concentration of phenolic compounds is in the extracts obtained using solvents of high and moderate polarity. The high dissolubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction [11, 12].

TABLE 1: TOTAL PHENOLIC CONTENTS IN THE PLANT EXTRACTS EXPRESSED IN TERMS OF GALLIC ACID EQUIVALENT (mg of ga/g of extract)

Test Samples	mg of GA/g of extract
DEP	0.32 ± 0.36
DEE	5.72 ± 0.29
DEM	15.32 ± 0.32

The concentration of flavonoids in DE whole plant extracts were determined using the spectrophotometric method with $AlCl_3$. The content of flavonoids is expressed in terms of Quercetin equivalent (QuE), mg of Qu/g of extract. The summary of quantities of flavonoids identified in the tested extracts is shown in **Table 2**.

TABLE 2: TOTAL FLAVONOID CONTENTS IN THE PLANT EXTRACTS EXPRESSED IN TERMS OF GALLIC ACID EQUIVALENT (mg of Qu/g of extract)

Test Samples	mg of Qu/g of extract
DEP	0.78 ± 0.49
DEE	5.14 ± 0.57
DEM	15.57 ± 0.69

The flavonoids content in the examined different extracts ranges from 0.78 to 15.57 mg Qu/g. The DEM extract (15.57) have very high values of total flavonoids contents when compared with DEP (0.78) and DEE (5.14). Based on the obtained values for the concentration of flavonoids in the observed leaf, bark and fruit extracts, it has been found that the highest concentration of these compounds is in the extracts obtained using solvents of moderate polarity and non-polar solvents. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation [13].

DPPH Radical Scavenging Activity: The comparison of the antioxidant activities of the extracts and ascorbic acid is shown in **Table 3**. The IC_{50} values for scavenging of free radicals were 35.28, 92.56, 334.82 and 457.51 µg/ml for ascorbic acid, methanol, ethyl acetate, and petroleum ether extracts respectively, which indicate the efficient DPPH scavenging activity. The order of DPPH scavenging activity was in the order of methanol>ethyl acetate > pet. ether extracts.

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY OF *DELONIX ELATA* EXTRACTS

S. no.	Conc. (µg/ml)	% Inhibition	IC_{50} (µg/ml)
Ascorbic Acid			
1	5	10.58	
2	10	25.26	
3	20	40.32	35.28
4	40	54.29	
5	80	66.34	
DEP			
1	25	16.58	
2	50	24.79	
3	100	36.08	457.51
4	200	40.52	
5	400	47.18	
DEE			
1	25	18.23	
2	50	20.46	
3	100	29.39	334.82
4	200	46.52	
5	400	52.08	
DEM			
1	25	18.32	
2	50	36.18	
3	100	51.29	92.56
4	200	59.36	
5	400	69.38	

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay: The comparison of the antioxidant activities of the extracts and ascorbic acid is shown in **Table 4**. The IC_{50} values for scavenging of free radicals were 56.35, 182.28, 304.33 and 397.26 µg/ml for ascorbic acid, methanol, ethyl acetate, and petroleum ether extracts respectively, which indicate the efficient H_2O_2 scavenging activity.

TABLE 4: HYDROGEN PEROXIDE (H_2O_2) SCAVENGING ASSAY OF *DELONIX ELATA* EXTRACTS

S. No.	Conc. (µg/ml)	% Inhibition	IC_{50} (µg/ml)
	Ascorbic Acid		
1	5	11.49	
2	10	26.67	
3	20	40.58	56.35
4	40	47.79	
5	80	64.38	
	DEP		
1	25	20.16	
2	50	25.38	
3	100	38.02	397.26
4	200	43.22	
5	400	50.48	
	DEE		
1	25	17.78	
2	50	20.68	
3	100	31.72	304.33
4	200	45.38	
5	400	55.49	
	DEM		
1	25	20.07	
2	50	29.38	
3	100	42.59	182.28
4	200	51.79	
5	400	75.52	

Nitric Oxide (NO) Radical Scavenging: The comparison of the antioxidant activities of the extracts and ascorbic acid is shown in **Table 5**.

TABLE 5: NITRIC OXIDE (NO) SCAVENGING ACTIVITY OF *DELONIX ELATA* EXTRACTS

S. no.	Conc. (µg/ml)	% Inhibition	IC_{50} (µg/ml)
	Ascorbic Acid		
1	5	21.72	
2	10	30.15	
3	20	37.47	67.97
4	40	46.25	
5	80	52.14	
	DEP		
1	25	23.47	
2	50	30.18	
3	100	32.29	386.49
4	200	41.32	
5	400	50.36	
	DEE		
1	25	27.38	
2	50	31.72	
3	100	38.09	176.83
4	200	54.27	
5	400	63.38	
	DEM		
1	25	21.74	
2	50	38.32	
3	100	47.56	143.88
4	200	54.31	
5	400	75.49	

The IC_{50} values for scavenging of free radicals were 67.97, 143.88, 176.83 and 386.49 µg/ml for ascorbic acid, methanol, ethyl acetate, and petroleum ether extracts respectively, which indicate the efficient NO scavenging activity.

Thiobarbituric Acid Reactive Substances (TBARS) Assay: The comparison of the antioxidant activities of the extracts and ascorbic acid is shown in **Table 6**. The IC_{50} values for scavenging of free radicals were 46.99, 121.94, 187.24 and 392.57 µg/ml for ascorbic acid, methanol, ethyl acetate, and petroleum ether extracts respectively, which indicate the efficient TBARS assay.

TABLE 6: THIOBARBITURIC ACID (TBARS) ASSAY OF *DELONIX ELATA* EXTRACTS

S. no.	Conc. (µg/ml)	% Inhibition	IC_{50} (µg/ml)
	Ascorbic Acid		
1	5	18.36	
2	10	27.21	
3	20	32.26	46.99
4	40	48.39	
5	80	65.44	
	DEP		
1	25	21.36	
2	50	35.46	
3	100	39.17	392.57
4	200	45.58	
5	400	50.51	
	DEE		
1	25	25.19	
2	50	31.36	
3	100	38.52	187.24
4	200	52.18	
5	400	62.56	
	DEM		
1	25	20.63	
2	50	35.19	
3	100	48.72	121.94
4	200	55.48	
5	400	70.78	

CONCLUSION: The *Delonix elata* whole plant methanolic extract showed a strong antioxidant activity by inhibiting DPPH, NO, H_2O_2, and TBARS activities when compared with the standard L-ascorbic acid. In addition, the *Delonix elata* was found to contain a noticeable amount of total phenols and flavonoids, which play a major role in controlling oxidation. The results of this study show that the *Delonix elata* whole plant can be used as an easily accessible source of natural

antioxidant. Therefore, a further study is needed to determine the mechanism behind the antioxidant activity of this plant. Further studies are needed to elucidate whether *Delonix elata* whole plant could be useful in the management of human diseases resulting from oxidative stress.

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CONFLICTS OF INTEREST: No conflicts of interests.

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