



## Anti-inflammatory and antioxidant activities of herbal decoction from the leaves of *Delonix elata* by *In vitro* condition

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### Abstract

The resolve of this study is to examine the effect of *Delonix elata* herbal decoction on antioxidant and anti-inflammatory activities. Total antioxidant and anti-inflammatory activities of herbal decoction from the leaves of *Delonix elata* were recorded and also phytochemical analysis was implemented. Moreover, inhibitory effects of inflammation related enzyme lipoxygenase and albumin denaturation were assessed and antioxidant 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), inhibition of lipid peroxidation and nitrite scavenging activities in a dose-dependent manner. The total phenolic and flavonoid contents of herbal decoction were  $61.23 \pm 2.67$  mg GAE/g and  $43.21 \pm 0.23$  mg QE/g, respectively. Treatment of 100  $\mu$ g/mL herbal decoction significantly reduced lipoxygenase (76.32%) and albumin denaturation (84.21%). The result of antioxidant activity ABTS radical (70.18%), inhibition of lipid peroxidation (77.38%) and nitrite scavenging (64.32%) activities dose-dependent manner. These results reinforce the fact that the antioxidant and anti-inflammatory activity of herbal decoction. Thus, herbal decoction from the leaves of *Delonix elata* may be utilized as a bioactive ingredient in functional foods.

**Keywords:** antioxidant, anti-inflammatory, *Delonix elata*, phytochemical analysis

### Introduction

Herbal plants are the richest bio resources of drugs of traditional system of medicine, modern medicine, nutraceuticals, food development, folk remedy, pharmaceutical intermediates and chemical creature for synthetic drugs. Remedial plants have been used in traditional treatment for several human diseases for thousands of years and they carry on being a vital remedial aid for recover the ailments of human kind (Momin and Kadam, 2011) [14]. The Herbal plants are helpful for healing as well as for curing of human diseases because of the existence of phytochemical molecules (Nostro *et al.*, 2000) [15]. Phytochemicals are naturally taking place in the herbal plants, leaves, vegetables and roots that have resistance mechanism and guard from various diseases. Phytochemicals are main and minor compounds. Chlorophyll, proteins and common sugars are integrated in primary constituents and main secondary compounds have terpenoid, alkaloids, flavonoids, steroids and saponins and phenolic compounds (Krishnaiah *et al.*, 2007) [11].

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Padmanabhan and Jangle, 2012) [17]. It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010) [21]. Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue (Denko.1992) [6].

To reach the total health care coverage of the world's population, traditional medicine is considered by WHO to be the most effective means since about 25% of modern prescription drugs are more or less obtained from plants. The severity and persistence of rheumatoid arthritis require long-term management with anti-inflammatory drugs. Nevertheless, these anti-inflammatory drugs have for the most part risks of toxicity for long-term use, which seriously limits their use. Current research in the management of rheumatoid arthritis is turning to a new generation of substances capable of selectively inhibiting TNF alpha and/or cyclooxygenase (COX-2) and having no major side effects. Recent interest in alternative treatments for arthritis favors the use of traditional medicine although scientific evidence of efficacy for most cases is lacking. Nevertheless, several herbs, used in a care program and a very effective preventive medicine, can act individually and/or in synergy to reduce chronic joint inflammation (osteoarthritis and/or rheumatoid arthritis). As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs (Kumarappan *et al.*, 2006) [12]. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs (Arivazhagan *et al.*, 2000) [2]. *Delonix elata* (L.) (Ceasalpinoidea) is a medium-sized tree that is widely distributed, in India. *D. elata* has been used in the Indian traditional medicine to treat rheumatism, inflammation, jaundice, and bronchial problems (Saravanan *et al.*, 2014). Decoction of the leaves and barks is taken to

get relief from rheumatic problems like pain and stiffness of the joints, especially affecting the knees. While in Saudi Arabia, all plant parts are used traditionally to relieve rheumatic pain, flatulence and the seeds are employed as purgatives. Previous biological studies on *D. elata* exhibited anti-inflammatory (Manimekalai *et al.*, 2011) [13], immune modifying potentials and antioxidant activities (Hegazi, 2011) [7].

## Materials and Methods

### Plant collection and preparation of extracts

*Delonix elata* leaves was obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-361/2021.

### Phytochemical Analysis

The aqueous extract of *Delonix elata* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973; Trease and Evans 1983).

### Thin Layer Chromatography

The herbal decoction from the leaves of *Delonix elata* were loaded on to pre coated TLC (60 F<sub>2</sub> 54) and it was developed using solvent system in the ratio of Petroleum ether, Chloroform and methanol (1:0.5:0.1, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F<sub>254</sub> (10x20 cm, 0.2mm layer). Visible and the non-visible spot given and it is fluorescent with UV light at 360nm and 240nm.

### Lipoxygenase Inhibition Assay

A spectrophotometric assay for determination of LOX activity was used as reported (Debnath *et al.*, 2013) with slight modification. Soybean 15-lipoxygenase (15-LOX) was used for the assay. Inhibition experiments were run by measuring the loss of soybean 15-LOX activity (5µg) with 0.2µM linoleic acid (Sigma) as the substrate prepared in solubilized state (Kemal *et al.*, 1987) in 0.2M borate buffer (pH 9.0). Inhibition studies in presence of various concentrations of herbal decoction of *Delonix elata* (25, 50, 75 and 100 µg/mL) and reference compound diclofenac sodium was recorded at 234 nm using UV-Vis spectrophotometer. The inhibitory effect of the extracts was also expressed as percentage of enzyme activity inhibition. EC<sub>50</sub> indicating the concentration required to inhibit 50 % LOX activity was also calculated. Values of hydroperoxide content and lipoxygenase activity were calculated from equation,

$$\text{Specific activity (LOX)} = \Delta A. V / \epsilon. l. c$$

Where,  $\Delta A$  is the value of absorbance increase per min, V is the volume of incubation mixture,  $\epsilon$  is the extinction coefficient for linoleic acid (25 x 10<sup>-3</sup> mol/l/cm), l is the length of the cuvette (1 cm) and c is the concentration of enzyme in mg (0.005). The values are mean of three independent experiments.

### IN-Vitro Protein Denaturation Inhibition Activity

*In vitro* anti-inflammatory activity of herbal decoction from the leaves of *Delonix elata*, the method used was "inhibition of protein denaturation" (Hmidani *et al.*, 2019) [8] using diclofenac sodium a standard. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (herbal decoction from the leaves of *Delonix elata*). The test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (25, 50, 75, 100µg/ml) of herbal decoction from the leaves of *Delonix elata* and diclofenac sodium (standard) were taken, respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm.

### Abts Radical Scavenging Activity

The ABTS scavenging activity was determined by the method of Re *et al.* (1999). The ABTS solution was produced by mixing 7.4 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with 2.6 mM potassium persulfate and then diluted with phosphate buffer saline (pH 7.4) until the absorbance of the ABTS solution was 0.70 ± 0.03 at 732 nm. After reacting 950 µL of diluted ABTS solution with 50 µL of herbal decoction from the leaves of *Delonix elata* for 10 min in the dark, the absorbance was measured at 732 nm using a spectrophotometer (Deep vision). The ABTS radical scavenging activity was calculated as the ratio of the decrease in absorbance between the sample and no sample.

### Inhibition of Lipid Peroxidation Activity

Lipid peroxidation induced by Fe<sup>2+</sup>ascorbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa *et al.* (1979). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO<sub>4</sub> (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·7H<sub>2</sub>O (0.06 mM); and different concentrations of the herbal decoction from the leaves of *Delonix elata* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV-Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to  $1 - (E/C) \times 100$ , where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

### Nitrite Scavenging Assay

The nitrite scavenging activity of each extract was measured according to the method of Kato *et al.* (1989) [9]. Specifically, the procedure was as follows: 1 mL of each concentration of samples was added to 2 mL of 1 mM NaNO<sub>2</sub> solution, and the pH of the reaction mixture was adjusted to 1.2 and 3.0 using 0.1 N HCl (pH 1.2) and 0.1 M citric acid buffer solution, respectively. This was followed by adjusting the final volume of the reaction mixture to 10 mL. Then, 1 mL of the reaction mixture obtained by reacting at 37°C for 1 h was taken, and 5 mL of 2% acetic acid was added, followed by mixing with 0.4 mL of Griess reagent. After incubating at room temperature for 15 min, the absorbance was measured at 520 nm using an absorption spectrophotometer. The nitrite scavenging activity was (%)

=  $[1 - (S - B)/C] \times 100$ , where C is the absorbance of the control, S is the absorbance of the sample without the Griess reagent, and B is the absorbance of the herbal decoction from the leaves of *Delonix elata* with the Griess reagent.

### Statistical Analysis

All experiments were performed in triplicates (n = 3) and the data are presented as the mean ± standard error. Differences between the means of the individual groups were analyzed using the analysis of variance procedure of SPSS software Version 20 (IBM).

### Result and Discussion

#### Phytochemical Screening

The phytochemical screening of the herbal decoction from the leaves of *Delonix elata* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and tannin (Table-1).

**Table 1:** Phytochemical screenings of herbal decoction from the leaves of *Delonix elata*

Sl. No.	Phytochemical Constituents	Observation	Herbal decoction from the leaves of <i>Delonix elata</i>
Alkaloids			
1	-Dragendorff's test	Orange/red precipitate	+
	-Mayers test	Cream pie PPT	+
Flavonoids			
2.	-Alkalai Reagent	Intense yellow colour	+
	-Lead acetate test	Precipitate formed	+
Glycosides			
3.	-Keller-Killiani test	Pink colour (Ammonia layers)	+
Tannin			
4.	-FeCl <sub>3</sub> test	Blue-black colour	+
Saponins			
5.	-Frothing test	Foam	-
Terpenoids			
6.	-Salkowski test	Reddish brown colour ring formed in interface	-
Polyphenols			
7.	-Ferrozine test	Raddish blue	+
Anthocyanin			
8.	-Ammonia test	Pink color in ammonia layer	+

+ Positive result; - Negative result

### The Partial Characterization of Herbal Decoction from the Leaves of *Delonix Elata* By TLC

The herbal decoction from the leaves of *Delonix elata* loaded on Pre-coated TLC plates (60 F<sub>2</sub> 54 Merck) and developed with a solvent system of petroleum ether, chloroform and methanol in the ratio of 1:0.5:0.1 were efficient to extract the anti-inflammatory and antioxidant compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Fig-1).

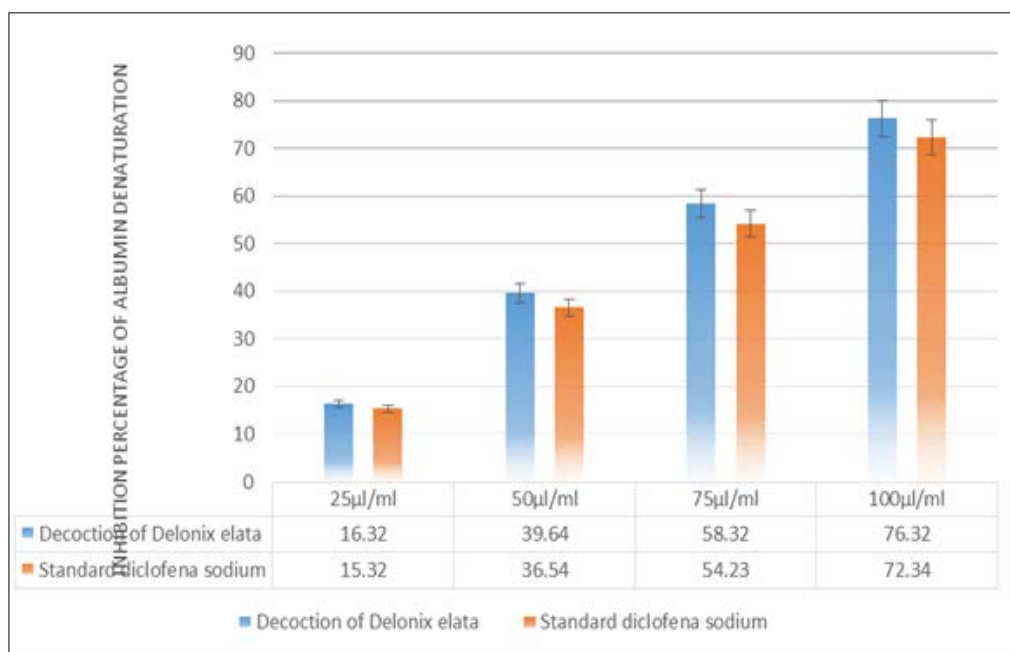
### Albumin Denaturation Inhibition

As fragment of the analysis on the mechanism of the anti-inflammatory activity, the capability to protein denaturation of the herbal decoction from the leaves of *Delonix elata* was recorded. It was effective in inhibiting albumin denaturation in Fig. 2. Maximum inhibition was recorded in the herbal decoction from the leaves of *Delonix elata* 76.31% at 100

µg/ml was. Diclofenac sodium was used standard drug, which showed the maximum inhibition of 76.32% at the concentration of 100 µg/ml. The EC<sub>50</sub> value of extract found to be 57.32 µg/ml which is higher than standard (Diclofenac sodium) value 63.24 µg/ml. Denaturation protein is recorded as reason of inflammation. Proteins denaturation is a well-documented cause of inflammation (Anoop and Bindu, 2015).

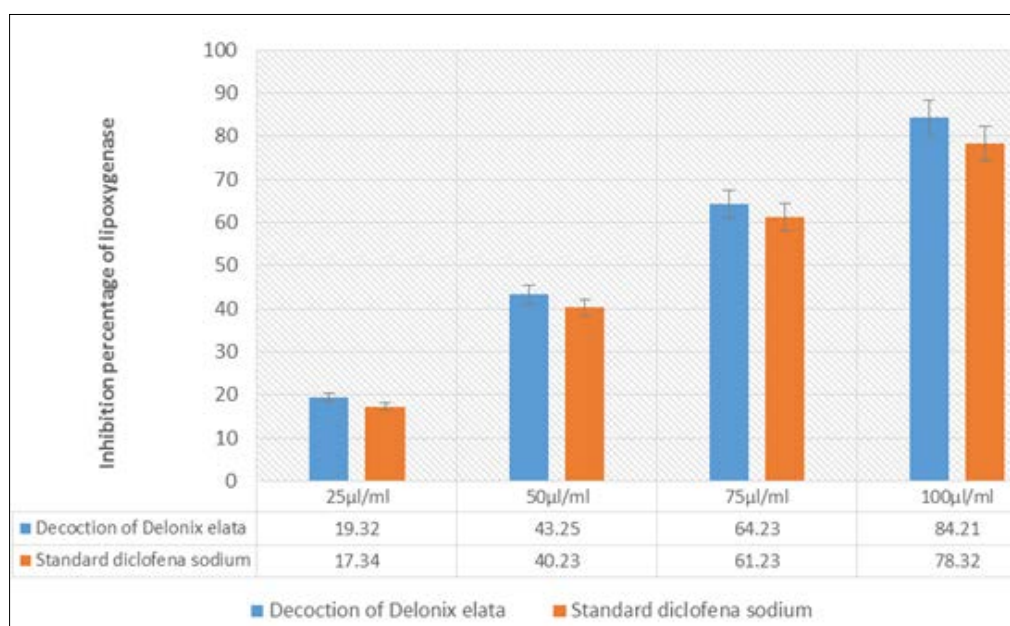
The inflammation mechanism involves a series of events in which the metabolism of arachidonic acid plays an important role.

The inhibitory activity of herbal decoction from the leaves of *Delonix elata* on the denaturation of proteins, the cyclooxygenase and 5-lipoxygenase pathways, and show that these two extracts are capable of significantly inhibiting the production of prostaglandins, which gives this extract anti-inflammatory properties.



**Fig 1:** Inhibition of Albumin Denaturation Herbal Decoction from the Leaves of *Delonix elata*

### Inhibition of 5-Lipoxygenase



**Fig 2:** Effect of herbal decoction from the leaves *Delonix elata* on inhibition of 5-lipoxygenase

The herbal decoction from the leaves of *Delonix elata* exhibited inhibitory activity of 5-Lipoxygenase compared to the standard diclofenac sodium. The herbal decoction from the leaves of *Delonix elata* recorded comparatively higher anti-inflammatory activity with regard to Lipoxygenase ( $EC_{50}$  56.32 µg/mL) than the standard (Fig-3). Most of these anti-inflammatory agents have validated and proved to be potential anti-inflammatory agents. In plants, compounds such as alkaloids, terpenoids and saponins have been found to be responsible to cure inflammatory disorders (Dawei *et al.*, 2004). In various inflammatory and allergic disorders, COX and 5-LOX are the main enzymes in the synthesis of prostanoids and eicosanoids from polyunsaturated fatty acids. The effective reduction of chronic inflammatory conditions is important by double inhibition of LOX (De Gaetano *et al.*, 2003) [4]. These results are justified by the

fact that herbal decoction from the leaves of *Delonix elata* possess an *In vitro* inhibition property of leukotriene B4 formation mediated by 5-LOX.

### Abts radical assay

The antiradical activity of the herbal decoction from the leaves of *Delonix elata*, as the demonstrative of nutritional food source, were evaluated *In vitro* by Substantial changes were witnessed for the polyphenol rich fraction, as well as between the assays employed. In table-2 the outcomes of antioxidant activity gained for tested samples, as well as Vitamin-C used as standard are presented. It can be evidently understood that polyphenol rich fraction exhibited prominent antioxidant activity, expressively higher than Vitamin-C.

**Table 2:** Free radical-scavenging ability using ABTS assay of the herbal decoction from the leaves of *Delonix elata*

Different concentration of extract	ABTS radical activity	
	The herbal decoction from the leaves of <i>Delonix elata</i>	Standard Vitamin-C
25 µl/ml	17.23±1.45	14.32±0.26
50 µl/ml	34.78±2.47	30.24±1.79
75 µl/ml	51.34±2.36	46.37±2.48
100 µl/ml	70.18±1.78	67.89±1.47
EC <sub>50</sub> value	66.32	71.38

<sup>a</sup> Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean±SD of three experiments

### Inhibition of Lipid Peroxidation

The herbal decoction from the leaves of *Delonix elata* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in herbal decoction from the leaves of *Delonix elata* (77.38%) and lowest inhibition percentage of Vitamin-C was found in 73.21% (Table-3). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). Phenolic and flavonoid compounds generally concentrated in the natural plant tissues include tannins, hydroxycinnamate esters, and lignins (Rice-Evans *et al.*, 1997) [19]. Additionally, notice in engaging antioxidants from natural sources to increase the shelf life of foods is considerably enhanced by consumer preference for natural constituents and concerns about the toxic effects of synthetic antioxidants (Kosar *et al.*, 2005) [10].

**Table 3:** Inhibition of lipid peroxidation activity of herbal decoction from the leaves of *Delonix elata*

Different concentration of extract	Inhibition of Lipid peroxidation activity	
	Herbal decoction from the leaves of <i>Delonix elata</i>	Standard Vitamin-C
25 µl/ml	20.34±0.79	19.34 ± 0.78
50 µl/ml	36.34±1.96	37.87 ± 1.89
75 µl/ml	57.23±0.89	55.32 ± 1.67
100 µl/ml	77.38±1.78	73.21 ± 2.49
EC <sub>50</sub> Value	57.12	63.21

<sup>a</sup> Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean±SD of three experiments.

### Nitric oxide radical scavenging assay

Nitric oxide radical quenching activity of herbal decoction from the leaves of *Delonix elata* were identified and compared with the standard ascorbic acid. The herbal decoction from the leaves of *Delonix elata* also caused a moderate dose-dependent inhibition of nitric oxide with an EC<sub>50</sub> of 83.21 µg/ml (Table-4). Vitamin-C was used as a reference compound and 87.21 µg/ml was needed for 50% inhibition. The EC<sub>50</sub> value of the extract was less than that of the standard. At 100 µg/ml, the percentage inhibition of the herbal decoction from the leaves of *Delonix elata* were 64.32% whereas that of Vitamin-C was 61.32%. Arbaayah and Umi (2013) [1] reported that the nitric oxide of the plant extracts can be due to the ability of hydrogens donation that stabilize the molecules by acceptance of hydrogen ions in the extracts.

**Table 4:** Nitric oxide radical scavenging assay of the herbal decoction from the leaves of *Delonix elata*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Herbal decoction from the leaves of <i>Delonix elata</i>	Ascorbic acid (+ve control)
25 µl/ml	16.32±2.34	15.34±1.34
50 µl/ml	29.64±0.48	27.34±2.79
75 µl/ml	42.31 ± 1.34	39.64 ± 1.48
100 µl/ml	64.32 ± 2.46	61.32 ± 2.48
EC <sub>50</sub> Value	83.21	87.21

<sup>a</sup> Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean±SD of three experiments.

### Conclusion

In conclusion, results specify that the herbal decoction from the leaves of *Delonix elata* possess anti-inflammatory and antioxidant properties at varying levels. Leaves of *Delonix elata* showed higher anti-inflammatory, inhibition activity of lipoxygenase and protein denaturation. Pearson's correlation studies showed that there were significant correlations between estimated anti-inflammatory and antioxidant properties. Results indicate that these anti-inflammatory and antioxidant activities may be due to the occurrence of bioactive saponin and flavonoid compounds in these leaves of *Delonix elata*.

### Acknowledgement

We thank Mr. SaiPrakash Leomuthu, CEO Sairam Institutions, Mr. SathishKumar CBO Sairam Institutions. Dr. S. Mathukumar M. D. (S), Principal Sri Sairam Siddha Medical College West Tambaram for Support and Encouragement to carry out the study.

### Reference

1. Arbaayah HH, Umi KY. Antioxidant properties in the oyster mushrooms (*Pleurotus* spp.) and split gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere*,2013;4:661-673.
2. Arivazhagan S, Balasenth S, Nagini S. Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium*. *Journal of Phytotherapy Research*,2000;14(4):291-293.
3. Bouhlali E.D.T., El Hilaly J., Ennassir J., Benlyas M., Alem C., Amarouch M.Y., Filali-Zegzouti Y. (2018). Anti-inflammatory properties and phenolic profile of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *J. King Saud Univ. Sci*,2018;30(4):519-526.
4. De Gaetano G, Donati MB, Cerletti C. Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors," *Trends in Pharmacological Sciences*,2003;24(5):245-252.
5. Debnath T, Park SR, Kim DH, Jo JE, Lim BO. Anti-oxidant and anti-inflammatory activities of *Inonotus obliquus* and germinated brown rice extracts. *Molecules*,2013;3:18(8):9293-9304.
6. Denko CW. A role of neuropeptides in inflammation. In: Whicher J T, Evans S W, eds. *Biochemistry in Inflammation*, ed. London: Kluwer Publisher,1992:177-181.
7. Hegazi GM. *In vitro* studies on *Delonix elata* L. an endangered medicinal plant. *WASJ*,2011;14:679-686.
8. Hmidani A, Bouhlali EDT, Khouya T, Ramchoun M, Filali-Zegzouti Y, Alem C, *et al.* Antioxidant, anti-

- inflammatory and anticoagulant activities of three Thymus species grown in southeastern Morocco. *Future. J. Pharm. Sci*,2019;5(1):4.
9. Kato H, Lee IE, Van Chuyen N, Kim SB, Hayase F. Inhibition of nitrosamine formation by nondialyzable melanoidins," *Agricultural and Biological Chemistry*,1987;51(5):1333-1338.
  10. Kosar M, Dorman HJD, Hiltunen R. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. *Food Chem*,2005;91:525-533.
  11. Krishnaiah D, Devi T, Bano A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants, *J. Medicinal Pl Research*,2009;3(2):67-72.
  12. Kumarappan CT, Chandra R, Mandal SC. Anti-inflammatory activity of *Ichnocarpus frutescens*. *Pharmacologyonline*,2006;3(2):201-206.
  13. Manimekalai K, Kartik JS, Harsha MS. Evaluation of the effect of the ethanolic extract of *Delonix elata* on acute inflammation in rats. *J. Natural Pharm*,2011;2:149-153.
  14. Momin RK, Kadam VB. Determination of ash values of some medicinal plants of genus *Sesbania* of marathwada region in maharashtra. *Journal of Phytology*,2011;3(12):52-54.
  15. Nostro A, Germano MP, D'Angelo A, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol*,2000;30(5):379-385.
  16. Ohkawa H, Ohisi N, Yagi K. Assay for lipid peroxides in animals tissue by thiobarbituric acid reaction. *Analytical Biochemistry*,1979;95:351-358.
  17. Padmanabhan P, Jangle SN. Evaluation of In-vitro Anti-inflammatory activity of herbal preparation, a combination of four medicinal plants,2012;2(1):109-116
  18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, *et al*. Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biology and Medicine*,1999;26(9-10):1231-1237.
  19. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. *Trends Plant Sci*,1997;2:152-159.
  20. Saravanan S, Hairul Islam VI, David HA, Lakshmi Sundaram R, Chellappandian M, Balakrishna K *et al*. Bioassay guided fractionation and identification of active anti-inflammatory constituent from *Delonix elata* flowers using RAW 264.7 cells. *Pharm. Biol*,2014;0:1-11.
  21. Umopathy E, Ndebia EJ, Meeme A, Adam B, Menziura P, Nkeh-Chungag BN *et al*. An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *Journal of Medicinal Plant Research*,2010;4(5):789-795.