

# IN VITRO ANTIOXIDANT ACTIVITIES OF AQUEOUS LEAF EXTRACT OF DELONIX REGIA

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## Abstract

**The present investigation assesses free radical scavenging action of aqueous extract of leaf of *Delonix regia* used in different in vitro models. The extract was subjected to qualitative and quantitative phytochemical screening. The extracts were utilized at 20, 40, 60, 80 and 100 µg / ml concentrations and radical scavenging activity was determined in terms of inhibition percentage, and the IC50 was likewise ascertained for every radical (using Ascorbic acid as the reference in the investigation). The study additionally revealed that aqueous extract of *Delonix regia* has high radical scavenging action in the different radical systems. Total phenolic content was 34.31 ± 0.169 mg Gallic acid equivalent (GAE) / g extract while flavonoid content was 55.765 ± 0.898mg Quercetin/g extract and total antioxidant capacity was 52.33 ± 1.443mg ascorbic acid equivalent. In conclusion, the antioxidant activity of *Delonix regia* aqueous leaf extract might be due to the high flavonoid and phenol content of the plant.**

**Keyword: *Delonix regia*, antioxidant, in vitro, antimutagenic, Lipid peroxidation assay**

## 1. INTRODUCTION

Antioxidants are substances which are fit for restraining a particular oxidizing enzymes or a substance that reacts with oxidizing agents prior to inducing damage to other molecules that sequesters metal ions or even a substance capable of rejuvenating system such as iron transporting protein [1]. Natural source is potential for

new medications; numerous plants have phytochemical properties which go about as antioxidants [2]. Oxidative stress is connected to inflammation, assuming together a critical part in the pathogenesis of malignancy [3], heart diseases [14], type 2 diabetes and corpulence [5]. Oxidative stress is a lopsidedness between generation of reactive oxygen species and antioxidant defenses [6]. The redox stress triggers the initiation of immune cells which release pro inflammatory cytokines, reactive oxygen and nitrogen species bringing harm to biomolecules and prompting imbalance in physiological and neurotic pathways [7]. Epidemiological and *in vivo* investigations have given proof that dietary consumption of antioxidants and anti-inflammatory compounds is a key procedure for wellbeing advancement by bringing down oxidative stress and inflammation [8].

Endogenous antioxidants are produced *in-vivo* in living organisms and repair free radical harm inside by starting cell recovery while exogenous antioxidants which are gotten from sources outside the living frameworks, for example, diets [9].

The developing need to supplement these endogenous antioxidants has prompted an expanded supplementation by exogenous sources. At present, there are unmistakable fascinations and far reaching researches on exogenous antioxidants from natural sources maybe, because of the way that they are more affordable, promptly accessible and accepted to have lesser symptoms when contrasted with their engineered partners [10].

*Delonix regia* (Hook) Raf is a type of little alluring tropical trees, it is regularly happening blooming plant

developed as an elaborate tree and given the name, colorful or fire tree, Gulmohar, peacock, Royal Poinciana. Numerous researchers have announced that blooms and green leaves of the *Delonix regia* are valuable as drugs [11,12]. The leaves are accounted for their antimicrobial and antioxidant impact [13]. It was reported *Delonix regia* is utilized in numerous nations for the preparation of extracts having antifungal and antimicrobial activities [14]. Be that as it may, till date there has been no details regarding the in-vitro antioxidant activities of the aqueous extract of the leaves of this plant which provoked this exploration. In this manner, this present work was embraced to determine the *in vitro* antioxidant activities and phytochemical constituents of aqueous leaf concentrate of *Delonix regia*.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

The leaves of *Delonix regia* were collected at the Federal Polytechnic Ilaro East Campus, Ilaro, Ogun state, Nigeria. The leaves were authenticated by a botanist at the Herbarium unit of Botany Department, University of Lagos. Voucher number LUH: **8043** was given and specimen samples was deposited at the herbarium.

### 2.2. Preparation of extracts

The fresh leaves were collected and dried at room temperature for 7days. The dried leaves were milled into fine powdered using mortar and pestle. Defined quantities of powdered dried leaves was macerated with 3% chloroform distilled water with occasional shaking for seven days to get aqueous extract. The aqueous extract was concentrated and dried using rotary evaporator. The extract was refrigerated until use.

$$\text{Percentage Yield \%} = \frac{\text{Weight of aqueous crude extract}}{\text{Weight of pulverized leaves}} \times 100$$

### 2.3. Phytochemical Analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plant under study were carried out in extracts using the standard procedures as described by [15-17].

### 2.4. Quantitative phytochemical analysis

**Total flavonoid content:** Estimation of the total flavonoids in the plant extract was carried out using the method of [18]. 1 ml of sample solution (100µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

**Total phenolic content:** Using modified Folin-Ciocalteu method [19], total phenol contents in the extracts were determined. 0.5g sample of extract was weighed and dissolved in 50 ml of water. 0.5 ml was added to 0.1 ml of Folin- Ciocalteu reagent (0.5N) and mixed and incubated at room temperature for 15 minutes. After this, 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

**Total antioxidant capacity determination:** Solution of the sample extract (1ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 °C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic.

### 2.5. Antioxidant Assay

Antioxidant assay were carried out on the aqueous extract of *D. regia* leaves as described by [20] and [21].

#### 2.5.1, 1 Diphenyl-2 picrylhydrazyl radical scavenging activity assay (DPPH)

An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was

mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the Expression: % inhibition =  $[A_0 - A_1] \times 100 / A_0$  (Where  $A_0$  is the absorption of the blank sample and  $A_1$  is the absorption of the extract).

### 2.5.2. *In vitro* lipid peroxidation assay

The reaction mixture containing liver homogenate (0.2ml), Tris-HCl buffer (20 mM pH 7.0, 0.1ml), FeCl<sub>2</sub> (2 mM, 0.1ml), ascorbic acid (10 mM, 0.1 ml), and 0.5 ml plant extract (25–100 µg/ml) in a final volume of 1 ml. The reaction mixture was incubated at 37 °C for 1 hour. Lipid peroxidation was measured as malondialdehyde (MDA) using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375 % w/v TBA; 15 % w/v TCA and 0.25 N HCl). The incubated reaction mixture was mixed with 2 ml of TBA-TCA reagent and heated in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 g for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. Ascorbic acid was used as standard.

### 2.5.3. Nitric oxide scavenging activity assay

4 ml sample of the aqueous extract or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% H<sub>3</sub>PO<sub>4</sub>). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylenediamine was measured at 550 nm. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$[A_0 - A_1] \times 100 / A_0$   
(Where,  $A_0$  is the absorbance of the Control and  $A_1$  is the absorbance of the extract or standard).

### 2.5.4. Reducing power assay

Various concentrations of the extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50c for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16µg/ml) was used as standard.

### 2.6. Statistical analysis

Results were expressed as the mean ± standard deviation. Data were subjected to analysis using Microsoft Excel and Graph Pad Prism 6, which was used to calculate IC<sub>50</sub> and to verify correlations between antioxidant parameters and total phenols and flavonoid content.

## 3. RESULTS

### 3.1. Percentage yield of the extract

Weight of the crude extract = 16.44g,  
weight of pulverized leaves = 130.62g  
% yield =  $\frac{16.44}{130.62} \times 100$   
% yield = 12.57

### 3.2. Qualitative phytochemical analysis

Phytochemical screening carried out showed that the aqueous extract of *Delonix regia* contains alkaloids, flavonoids, glycosides, phenol, terpenoids, amino acids, steroids, saponins, diterpenes, steroids, tannins and reducing sugar. The results are given in Table 1 below.

### 3.3. Quantitative phytochemical analysis Total flavonoids, Total phenol and Total antioxidant capacity

The results in table 2 below, show the total phenolic contents of *D. regia* expressed as µg/mg gallic acid equivalent, total flavonoids expressed as quercetin and antioxidant capacity was expressed as equivalent of ascorbic. Total flavonoids fraction had the highest total capacity 55.765±0.898mg followed by antioxidant capacity (Ascorbic) 52.33±1.443mg and phenolic contents 34.31±0.169ng (GAE). The extract is a good source of Flavonoids and Antioxidant compounds.

Flavonoids	+
Tannin	+
Saponin	+
Phenol	+
Diterpenes	+
Amino acids	+
Glycosides	+
Steroids	+
Reducing sugar	+
Terpernoids	+

+ present, -absent

**Table 1: Phytochemical analysis of aqueous extract of leaves of *Delonix regia***

Phytochemical	Result
Alkaloids	+

**Table 2: Quantitative phytochemical analysis**

Phenolics	Extract
Total phenolic content <sup>a</sup>	34.31 ± 0.169 mg
Total flavonoids <sup>b</sup>	55.765 ± 0.898 mg
Total antioxidant capacity <sup>c</sup>	52.33 ± 1.443 mg

<sup>a</sup>Expressed as mg of gallic acid equivalent (GAE)/g of dry plant material.

<sup>b</sup>Expressed as mg quercetin/g of dry plant material.

<sup>c</sup>Expressed as equivalent of ascorbic acid/g of dry plant material

### 3.4. Antioxidant Assay

**1, 1-Diphenyl- 2 picrylhydrazyl free radical scavenging ability (DPPH):** The aqueous leaves extracts exhibited 37.805 ± 0.177 and 85.565±0.361 percent inhibition, at the concentration of 20µg/ml and 100µg/ml respectively, whereas the percentage inhibition values of Ascorbic acid were found to be 46.46±0.353 and 83.245±0.728 percent, at the concentration of 20µg/ml and 100µg/ml respectively. The DPPH radical scavenging activity values of the extract along with standard Ascorbic acid were shown in Figure 1. The IC<sub>50</sub> value of the extract was found to be 41.403µg/ml, whereas IC<sub>50</sub> value of Ascorbic acid was 20.432µg/ml. As shown in Figure 1, DPPH Scavenging assay of the extract increases in a concentration-dependent manner as exhibited by the standard, ascorbic acid. However, the extract was moderately good compared to ascorbic acid.

**Nitric oxide scavenging activity:** The aqueous crude extract of leaves of *D. regia* showed significant scavenging activity. The percentage inhibition values of aqueous leaves extract were found to be 35.6±0.127 and 74.07±0.311 at the concentration of 20 and 100µg/ml respectively. The percentage inhibition values of Ascorbic acid were found to be 43.32±0.651 and 88.06±0.495 percent at concentrations of 20 and 100µg/ml respectively (Figure 2).

The IC<sub>50</sub> value of *D. regia* leaves extract was 53.241µg/ml. Whereas IC<sub>50</sub> value of ascorbic acid was 32.540µg/ml. There was an increase in the Nitric oxide radical due to the scavenging ability compared to ascorbic acid; this observation further shows that the extract can slightly prevent the physiological deleterious caused by nitric oxide free radical.

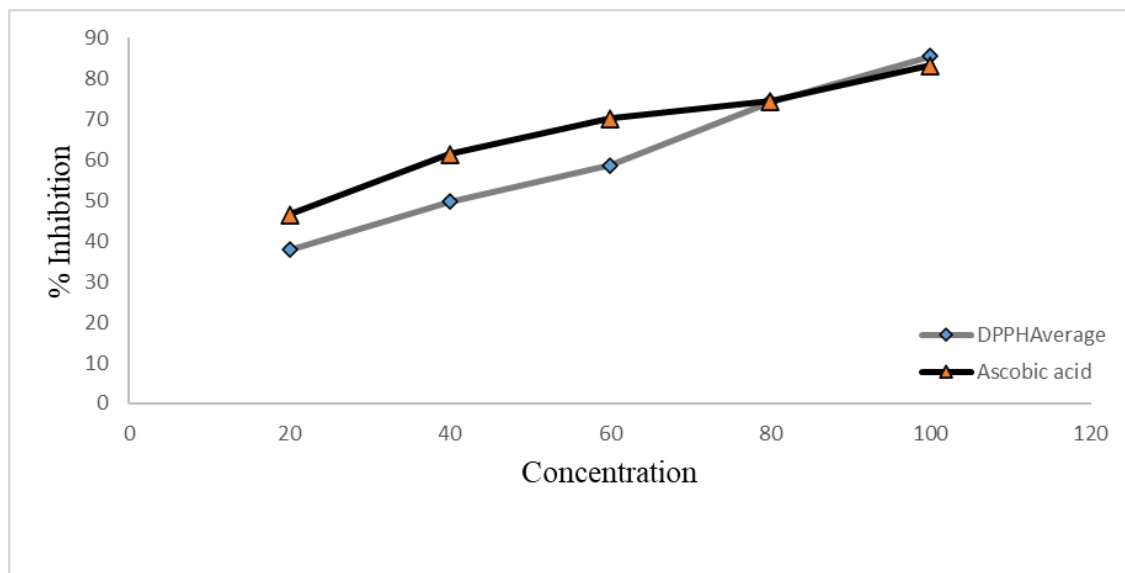


Figure 1: DPPH scavenging activity

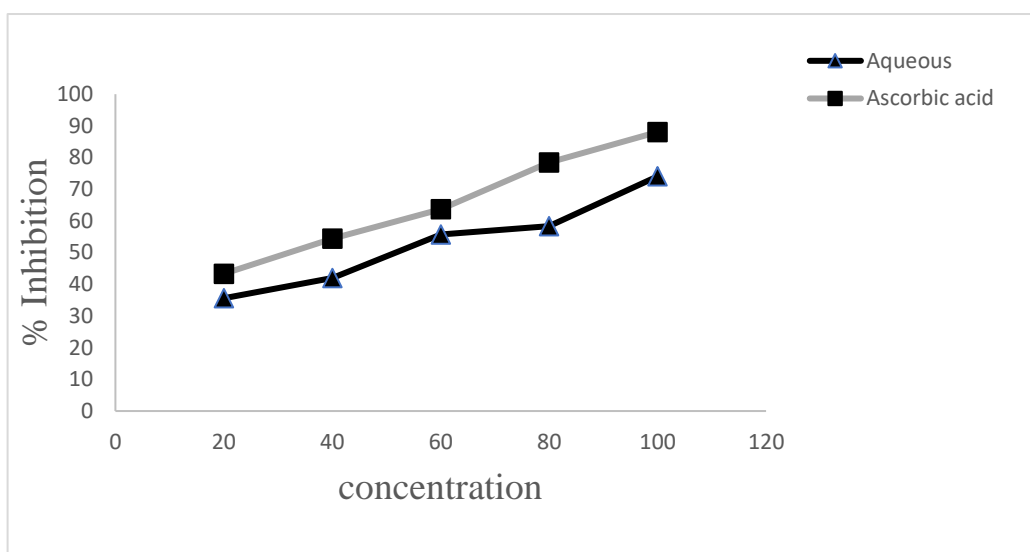


Figure 2: Nitric oxide scavenging activity

**Reducing power assay:** The IC<sub>50</sub> value of the extract was 13873.67µg/ml while IC<sub>50</sub> value of ascorbic acid was 8045.032µg/ml. However, its reducing power was weaker than that of ascorbic acid, which exhibited stronger reducing power. Therefore, antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and non-reactive species.

**Lipid peroxidation scavenging activity:** The percentage inhibition of lipid peroxidation in aqueous leaves extract was significantly lower when compared with ascorbic acid (standard) as shown in figure 4. The IC<sub>50</sub> value of the extract was 55.51257µg/ml while the IC<sub>50</sub> value of ascorbic acid was 23.62924µg/ml.

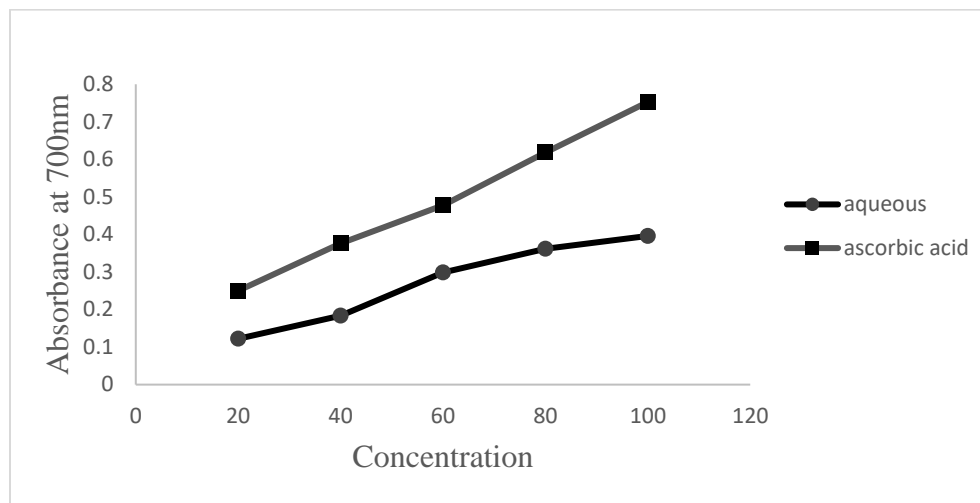


Figure 3: Reducing power scavenging activity

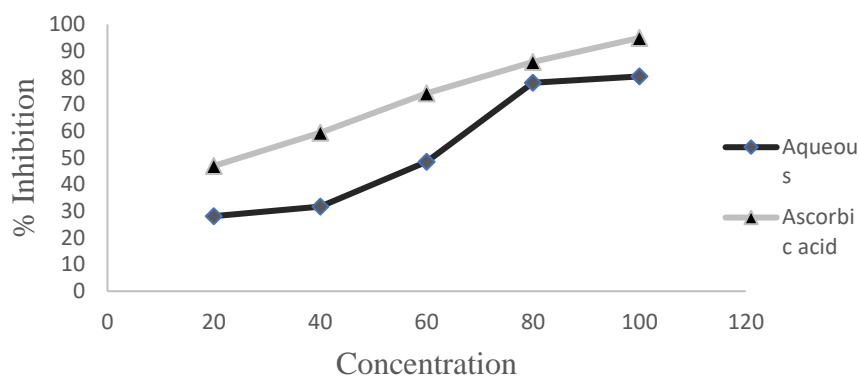


Figure 4: Lipid peroxidation scavenging activity

#### 4. DISCUSSION

Plants have varying group of phenolic compounds, for example, simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid subsidiaries and flavonoids. All these phenolic classes have increased broad consideration in view of their physiological capacities, including free radical scavenging, antimutagenic, anti-carcinogenic and anti-inflammatory impacts [22,23].

[24] detailed that the antioxidant effect of phenolics is to a great extent because of their redox properties which influence them to go about as reductants,

hydrogen donors, singlet oxygen quenchers and also potential metal chelators. In this investigation, a significant high quantity of phenolics was seen in the aqueous extract of the leaf of *Delonix regia*. This may clarify the across the board legends utilization of the plant.

[25], revealed the presence of tannin, terpenoid, flavonoids, steroids and fatty acids in the chloroform extract of *Delonix regia* leaves. Alkaloid, phlobatannin and saponin were also reported to be present. The chloroform extract of *D. regia* likewise demonstrated mild antioxidant activity in DPPH, hydrogen peroxide and reducing power assay systems which isn't absolutely

in concurrence with the discoveries of this study. This proposes the aqueous extract of *D. regia* may contain a greater number of phytochemicals than the chloroform extricate.

DPPH is a steady free radical and accepts an electron or hydrogen radical and becomes a stable diamagnetic atom which is generally used to explore radical scavenging activity. In DPPH radical scavenging assay, antioxidants react with DPPH (profound violet shading) and change over it to yellow hued  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picryl hydrazine. The level of staining shows the radical-searching capability of the antioxidant [26]. In the present examination, there was increased scavenging activity of the DPPH radicals with increasing concentration of the plant extract which may show an expanded capacity to give hydrogen particles bringing about a lighter arrangement which is relative to the quantity of electrons picked up [27,28]. Thusly, it might be hypothesized that the fluid leaf concentrate of *Delonix regia*, has DPPH scavenging activity.

Nitric oxide radical assumes various parts in an assortment of natural processes which fills in as an effector molecule, neuronal messenger vasodilator and antimicrobial agent [29]. It has been accounted for to react with  $\bullet\text{O}_2$  radical to shape peroxy radicals (ONOO-) that brings about toxic action on biomolecules, for example, proteins, lipids and nucleic acids [30]. During the inflammatory process, cells of the immune system produce superoxide radicals in which NADPH oxidase assumes a critical part in induction of vascular entanglements [31].  $\bullet\text{O}_2$  additionally deteriorates into singlet oxygen and HO $\bullet$  that outcome in gigantic mitochondrial harm. The result recommended that nitric oxide scavenging power of the extract was dependent on concentration.

The decreasing capacity of a compound by and large relies upon the presence of reductants [32] which have been displaying antioxidative potential by breaking the free radical chain and giving a hydrogen atom [33]. The extract demonstrated good reducing power capacity in a dose dependent way which was equivalent to that of standards. The antioxidant principles present in the extract of *D. regia* caused the decrease of Fe<sup>3+</sup> / ferricyanide complex to the ferrous form and accordingly demonstrated the reducing power capacity. Lipid peroxidation is an amassed impact of reactive oxygen species (ROS), which prompts decay of biological frameworks. It might be started by reactive

free radicals, which subtract an allylic hydrogen molecule from a methylene gathering of polyunsaturated fatty acid side chains. This is joined by bond adjustment that outcomes in stabilization by diene conjugate formation. The lipid radical at that point takes up oxygen to frame peroxy species [34].

The present examination has uncovered that the aqueous of *D. regia* contains significant measure of phenolics and accordingly, can be gathered that these phenolics are in charge of its stamped antioxidant activity as tested through different in vitro models utilized in this investigation. This is predictable with a few reports that have indicated cozy connection between total phenolic content and antioxidant activity of natural products, plants and vegetables [35-37].

## 5. CONCLUSION

In this study, it may be concluded that the aqueous leaf extract of *Delonix regia* was observed to be a rich source of phytochemicals. It can likewise be gathered that aqueous extract of *D. regia* leaves has respectably great antioxidant activity which could be credited to the presence of flavonoids, alkaloids, tannins, saponin glycosides and phenolic compounds. In this manner the leaves of *D. regia* can be useful as an effortlessly available source of antioxidants and as a conceivable nourishment supplement or in pharmaceutical industry.

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## 7. CONFLICT OF INTEREST

Authors declare there is no conflict of interest associated with the research.

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