



IN VITRO* ANTIOXIDANT ACTIVITY OF AQUEOUS LEAF EXTRACT OF *Delonix regia

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ABSTRACT

Free radicals are reactive particles engaged with numerous physiological processes and have been related with numerous illnesses. Therefore, there is need to investigate substances with free radical scavenging ability. The present investigation was intended to assess the free radical scavenging action of aqueous extract of leaf of *Delonix regia* utilizing different in vitro models. The extract was subjected to qualitative and quantitative (total phenol, total flavonoid and total antioxidant capacity) phytochemical screening. Ascorbic acid was the reference in the investigation. 1,1-Diphenyl-2-picryl hydroxyl (DPPH) and nitric oxide scavenging assays, as well as reducing power assay were carried out in this analysis. The capacity of the extracts to restrain lipid peroxidation was estimated by the in vitro lipid peroxidation assay. The extracts were at 20, 40, 60, 80 and 100 µg/mL concentrations and radical scavenging activity was determined in terms of inhibition percentage. The IC₅₀ (concentration required for 50 % inhibition) was likewise ascertained for every radical. Qualitative phytochemical screening uncovered the presence of alkaloid, flavonoid, tannin, saponin, diterpenes, phenol, amino acids. The study additionally uncovered that the aqueous extract of *Delonix regia* leaf has a high radical scavenging action in the different radical systems. The total phenolic content and, flavonoid contents were 34.31 ± 0.169 mg Gallic acid equivalent (GAE)/g extract and 55.765 ± 0.898 mg Quercetin/g extract respectively while the total antioxidant capacity was 52.33 ± 1.443 mg ascorbic acid equivalent. The antioxidant activity of *Delonix regia* aqueous leaf extract could be related to high flavonoid and phenol content of the plant.

Keywords: *Delonix regia, total phenol, total flavonoid, DPPH, Nitric oxide*

INTRODUCTION

Antioxidants and cancer prevention agent 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 (Brewer 2011). Natural source is potential for new medications; numerous plants have phytochemical properties which go about as antioxidants (Rudy *et al.*, 2006). Oxidative stress is connected to inflammation, assuming together a critical part in the pathogenesis of malignancy (Maeda and Omata, 2008), heart diseases (Montenucco *et al.*, 2011), type 2 diabetes and corpulence (Sell and Eckel, 2009). Oxidative stress is a lopsidedness between generation of reactive oxygen species and antioxidant defenses (Betteridge, 2000). 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 (Lonkar and Dedon, 2011). 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 (Watz, 2008).

Endogenous antioxidants are produced in vivo in living organisms and repair free radical harm inside by starting cell recovery while exogenous cell reinforcements which are gotten from sources outside the living frameworks, for example, diets (Jaouad and Torsten, 2010).

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 (Tadhani *et al.*, 2007).

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 (Koshimizur *et al.*, 1988). The leaves are accounted for their antimicrobial and antioxidant impact (Aly *et al.*, 2011). It was reported *Delonix regia* is utilized in numerous nations for the preparation of extracts having antifungal and antimicrobial activities (Sammour and El-Shanshoury,

1982). 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 the aqueous leaf extract of *Delonix regia*.

MATERIALS AND METHOD

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 and specimen samples was deposited at the herbarium.

Preparation of extracts

The fresh leaves were collected and dried at room temperature for 7 days. The dried leaves were milled into fine powdered using mortar and pestle. Powdered dried leaves (130.64g) and 1L of distilled water (Aqueous) were stoppered in a container and soaked for 24 hours (Maceration Method). After the extraction, the mixture was filtered through muslin cloth and the filtrate solution was concentrated in a rotary evaporator to get the Crude extract. The Aqueous crude extract was weighed and refrigerated prior to further analysis.

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

Phytochemical analysis

Chemical tests for the screening of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using the standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973). Alkaloids, phenols, tannins, flavonoids, amino acids, diterpenes, saponins, glycoside, terpenoids, reducing sugar, steroids were qualitatively screened.

Total flavonoid content

Estimation of the total flavonoids in the plant extract was carried out using the method of Ordonez *et al.* (2006). Sample solution 1 mL (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 compound

Using modified Folin–Ciocalteu method, total phenol contents in the extracts was determined. Sample extract (0.5g) was weighed and dissolved in 50 mL of water. A portion (0.5 mL) was added to 0.1 mL of Folin-Ciocalteu reagent (0.5 N) and mixed and incubated at room temperature for 15 minutes. Immediately after, 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 (1 mL) 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

Antioxidant assay

Antioxidant assay were carried out on the aqueous extract of *D. regia* leaves as described by Deepa *et al.*, (2013) and Jelili *et al.*, (2011).

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, 100 µg/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

In vitro lipid peroxidation assay

The reaction mixture containing liver homogenate (0.2 mL), Tris–HCl buffer (20 mM pH 7.0, 0.1 mL), $FeCl_2$ (2 mM, 0.1 mL), 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.

Nitric oxide scavenging activity assay

Sample solution (4 mL) 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% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthylethylene diamine 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.

Reducing power assay

Various concentrations of the extracts (20 to 100 µg/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 50°C 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 16 µg/mL) was used as standard.

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 IC50 and to verify correlations between antioxidant parameters and total phenols and flavonoid content.

RESULTS AND DISCUSSION

The percentage yield (12.57%) of the plant under study proves that the plant possess high potential source for the phyto compounds. The yield base on the polarity of the solvent an indication of the plants' pharmacological importance.

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 were given in Table 1.

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 (Bandoniene and Murkovic, 2002).

Mariajancyriani *et al.*, (2013), 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.

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

Phytochemical	Result
Alkaloids	+
Flavonoids	+
Tannin	+
Saponin	+
Phenol	+
Diterpenes	+
Amino acids	+
Glycosides	+
Steroids	+
Reducing sugar	+
Terpenoids	+

+ presence - absence

Total Flavonoids, Total Phenol and Total Antioxidant Capacity.

The results in table 2 show the total phenolic contents of *D.regia* expressed as $\mu\text{g}/\text{mg}$ gallic acid equivalent, total flavonoids expressed as quercetin and antioxidant capacity was expressed as equivalent of ascorbic. Total flavonoids was found to be $55.765 \pm 0.898 \text{ mg mg quercetin/g}$ of dry plant material. Total antioxidant capacity (Ascorbic) was $52.33 \pm 1.443 \text{ mg equivalent of ascorbic acid/g}$ of dry plant material and phenolic contents $34.31 \pm 0.169 \text{ ng (GAE)}$.

Pietta, (2000) 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.

Table 2: Quantitative phytochemical analysis

Phenolics	Extract
Total phenolic contenta	34.31 ± 0.169
Total flavonoidsb	55.765 ± 0.898
Total antioxidant capacityc	52.33 ± 1.443

aExpressed as mg of gallic acid equivalent (GAE)/g of dry plant material.

bExpressed as mg quercetin/g of dry plant material.

cExpressed as equivalent of ascorbic acid/g of dry plant material

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 $20 \mu\text{g}/\text{ml}$ and $100 \mu\text{g}/\text{mL}$ 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 $20 \mu\text{g}/\text{mL}$ and $100 \mu\text{g}/\text{mL}$ respectively. The DPPH radical scavenging activity values of the extract along with standard Ascorbic acid were shown in Figure 1. The IC50 value of the extract was found to be $41.403 \mu\text{g}/\text{mL}$, whereas IC50 value of Ascorbic acid was $20.432 \mu\text{g}/\text{mL}$. Low IC50 values are indicative of a very positive radical scavenging effect of the extract. 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.

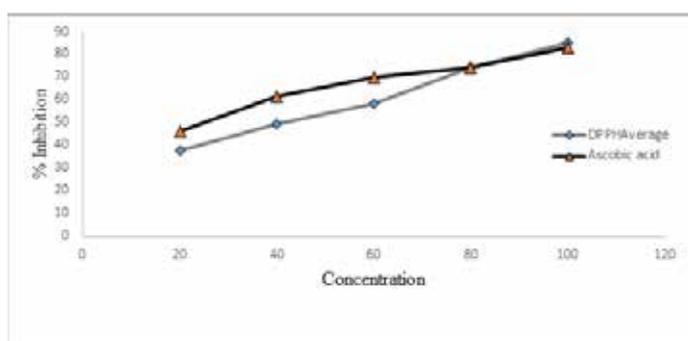


Figure 1: DPPH Scavenging assay

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 α , α -diphenyl- β -picryl hydrazine. The level of staining shows the radical-searching capability of the antioxidant (Blois, 1958). 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 (Villano *et al.*, 2007). Thusly, it might be hypothesized that the fluid leaf concentrate of *Delonix regia*, has DPPH scavenging activity.

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 100 $\mu\text{g/mL}$ 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 100 $\mu\text{g/mL}$ respectively (Figure 2).

The IC₅₀ value of *D. regia* leaves extract was 53.241 $\mu\text{g/mL}$. Whereas IC₅₀ value of ascorbic acid was 32.540 $\mu\text{g/mL}$.

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.

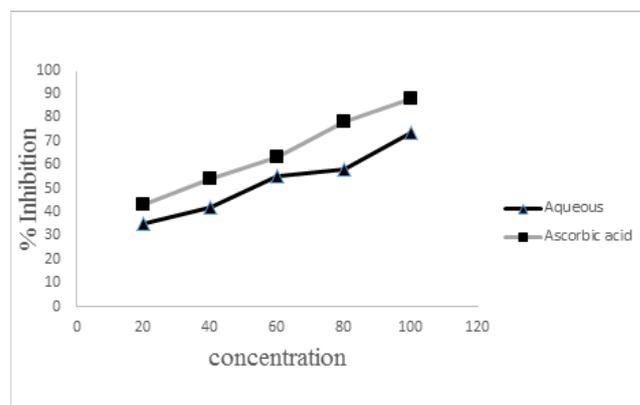


Figure 2 Nitric oxide scavenging activity

Nitric oxide radical assumes various parts in an assortment of natural processes which fills in as an effector molecule, neuronal messenger vasodilator, antimicrobial agent (Hagerman *et al.*, 1998). It has been accounted for to react with $\cdot\text{O}_2$ radical to shape peroxy nitrite radicals (ONOO) that brings about toxic action on biomolecules, for example, proteins, lipids and nucleic acids (Yermilov *et al.*, 1995). 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 (Droge, 2002). $\cdot\text{O}_2$ additionally deteriorates into singlet oxygen and $\text{HO}\cdot$ that outcome in gigantic mitochondrial harm. The result recommended that nitric oxide scavenging power of the extract was dependent on concentration.

Reducing power assay

The IC₅₀ value of the extract was 13873.67 $\mu\text{g/mL}$ while IC₅₀ value of ascorbic acid was 8045.032 $\mu\text{g/mL}$. 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.

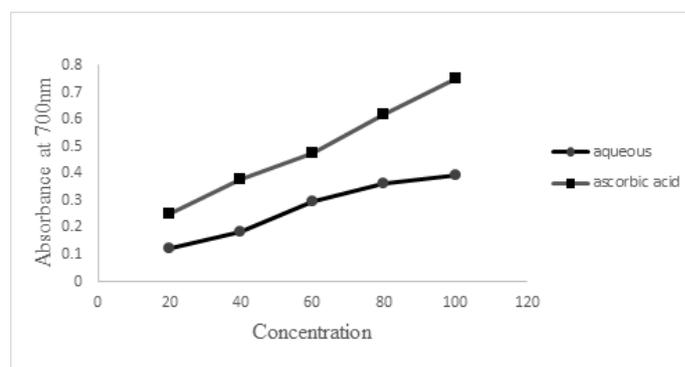


Figure 3 Reducing power scavenging assay

The decreasing capacity of a compound by and large relies upon the presence of reductants (Duh *et al.*, 1999) which have been displaying antioxidative potential by breaking the free radical chain and giving a hydrogen atom (Gordon 1990). 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 Fe³⁺/ferricyanide complex to the ferrous form and accordingly demonstrated the reducing power capacity.

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 IC₅₀ value of the extract was 55.51257 µg/ml while the IC₅₀ value of ascorbic acid was 23.62924 µg/ml.

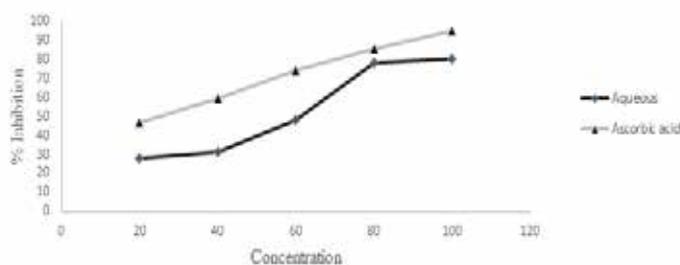


Figure 4 Lipid peroxidation scavenging assay

REFERENCES

- Aly, M., E., Ezzat, S. M., Maha, M. S. & Amany, A. S. (2011). Hepatoprotective and Cytotoxic Activities of *Delonix regia* Flower Extracts, *Phycology Journal*. 3(19); 49-56
- Baba, S.A and Malik, A.S. (2015). Determination of total phenolic and flavonoid content, anti-microbial and antioxidant activity of a root extract of *Arisaema jacquemonti* Blume. *J. Taibah Univ. Science*. 9: 449–454.
- Bandoniene, D and Murkovic, M. (2002). On-line HPLC-DPPH screening method for evaluation of radical scavenging phenols extracted from apples (*Malus domestica* L.). *J. Agric. Food Chemistry*. 50: 2482–2487.
- Betteridge, D.J. (2000). What is oxidative stress?. *Metabolism*. 49(2): 3-8.
- Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*. 181: 1199–1200.
- Brewer M.S. (2011). Natural antioxidants: sources, compounds, mechanisms of action and potential applications. *Food Safety*. 10: 221–246.
- Deepa, B., Prema, G., Sai, K., Borra, C and Cherian, K.M. (2013). Antioxidant and free radical scavenging activity of triphala determined by using different in vitro models. *International journal*. 7(39): 2898-2905.
- Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Revision*. 2002; 82: 47-95.
- Duh, P.D, Tu, Y.Y and Yen, G.C. (1999). Antioxidant activity of the aqueous extract of harn jyur (*Chrysanthemum morifolium* Ramat). *Lebensmittel-Wissenschaft and Technologies*. 32: 269-277.
- Dzingiral, B., Muchuweti, M., Murenje, T., Chidewe, C., Benhura, M.A.N., Chagonda, L.S (2007). Phenolic content and phospholipid peroxidation inhibition by methanolic extracts of two medicinal plants: *Elionurus muticus* and *Hpoxis hemerocallidae*. *African Journal Biochemistry Resources*. 1: 137.

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 (Dzingiral *et al.*, 2007).

The present study 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 (Baba and Malik, 2015).

CONCLUSION

In this study, it very well 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 the leaves has great antioxidant activity which could be credited to the presence of flavonoids, alkaloids, tannins, saponins, glycosides and phenolic compounds. Thus, 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.

- Gordon, M.H. (1990). The mechanism of antioxidant action in vitro. In: B.J.F. Hudson (Ed.), *Food antioxidants Elsevier Applied Science*, London, 1–18.
- Hagerman, A.E., Jones, K.M., Riedl, S.G.A. (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal Agric. Food Chemistry*. 46: 1887-1892
- Harborne, J.B. (1973). *Phytochemical methods*, London. Chapman and Hall, Ltd. pp. 49-188
- Jaouad, B., Torsten, B. (2010). Exogenous antioxidants—Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. *Oxid Med Cell Longevity*. 3(4): 228–237.
- Jelili, A., Temitope, O., John, O., Victor, A., Oluwatosin, A., and Oyeronke, A. (2011). Lipid peroxidation inhibition and antiradical activities of some leaf fractions of mangifera indica. *Acta Poloniae Pharmaceutica ñ Drug Research*. 68(1) 23-29
- Kaur, C., Kapoor, H.C. (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technology*, 37, 153–161.
- Koshimizur, K., Ohigashi-Tokuda, H., Kondo, A., and Yamaguchi, K. (1988). Screening of Edible Plants against Possible Antitumor Promoting Activity. *Cancer Lett*. 39: 247
- Lonkar, P., Dedon, P.C. (2011). Reactive species and DNA damage in chronic inflammation: reconciling chemical mechanisms and biological fates. *International. Journal Cancer*. 128(9): 1999-2009.
- Maeda, S., Omata, M. (2008). Inflammation and cancer: role of nuclear factor-kappa B activation. *Cancer Science*. 99(5): 836-842.
- Mariajancyrani, J., Chandramohan, G., Saravanan, P., Saivaraj, S. (2013). In-Vitro Antioxidant Potential of *Delonix regia* Leaves. *Sch. Acad. J. Pharmacy*. 2(6):468-471.
- Montecucco, F., Pende, A., Quercioli, A., Mach, F. (2011). Inflammation in the pathophysiology of essential hypertension. *Journal Nephrology*. 24(1): 23-34.
- Ordóñez, A.A.L., Gomez, J.D., Vattuone, M.A., Isla, M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry*. 97: 452–458.
- Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of Natural Products*. 63: 1035–1042.
- Rudy, J.C., Hyoung-gon, L., Goerge, P., Mark, A.S. (2006). Alzheimer Disease Pathology as a Host Response. *American Journal of Alzheimers Disease and Other Dementias*. 21(2).
- Sammour, R.H., El-Shanshoury, A.E.R. (1992). Antimicrobial activity of legume seed proteins. *Bot. Bull. Acad. Sin*. 33: 185-190.
- Sell, H., Eckel, J. (2009). Chemotactic cytokines, obesity and type 2 diabetes: in vivo and in vitro evidence for a possible causal correlation? *Process Nutrition*. 68(4):378-384.
- Sofowara, A. (1993). *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. 1993 p. 289.
- Tadhani, M.B., Patel, V.H., Subhash, R. (2007). In vitro antioxidant activities of *Stevia rebaudiana* leaves and callus. *J. Food Comp. Analysis*. 20: 323–329.
- Trease, G.E., Evans, W.C. (2005). *Pharmacognsy*. 11th edn. Brailliar Tiridel Macmillian publishers. 21(2)
- Villaño, D., Fernández-Pachón, M.S., Moyá, M.L., Troncoso, A.M., García-Parrilla, M.C. (3007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*. 71 (1): 230–235.
- Watz, B. (2008). Anti-inflammatory effects of plant-based foods and of their constituents. *International Journal Vitam Nutrition Resource*. 78(6): 293-29.
- Yermilov, V., Rubio, J., Becchi, M., Friesen, M.D., Pignatelli, B., Ohshima, H. (1995). Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite in vitro. *Carcinogenesis*. 16(9):2045-50.