*Proceedings S.Z.M.C. Vol: 39(2): pp. 102-105, 2025.* *PSZMC-980-39-2-2025*

# Antioxidant Properties of n-Hexane and Acetone Leaf Extracts of *Delonix regia* (گل موہر )

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**Received:** Feb 4, 2024, **Accepted:** June 20, 2025, **Published:** June 30, 2025.
DOI: https://doi.org/10.47489/szmc.v39i2.716
**Data Availability:** The corresponding author can provide access upon request.
**Funding:** No funding received
**Competing Interests:** No competing interest to declare.

**Author Contributions:**
SAS, Study conception and design, perform all experiments, writing. AA, Study conception and design, supervision. A, Experimentation and data collection. SNA, Guided in research work and manuscript writing. FJ, Manuscript writing. SN, Manuscript writing, review, and proofreading.
**Academic Editor:**
Prof. Dr Mudassira Saqib *(MBBS, MPhil, PhD),* Pharmacology and Therapeutics, Shaikh Zayed Postgraduate Medical Institute, Lahore, Pakistan.

## ABSTRACT

**Background:** *Delonix regia*, commonly known as Gulmoharگلموہر, is a tropical flowering tree from the pea family, widely found in tropical regions. In traditional folk medicine, its leaves have been employed to cure numerous diseases. In contemporary times, there is an emergent trend among individuals to prioritize their health, often looking for new products derived from nature whereas avoiding synthetic alternatives. Plants are primary source of bioactive compounds, including natural antioxidants, which are essential in drug development and the formulation of healthcare products.
**Objective:** To assess the antioxidant property of leaf extract of *D. regia* in acetone and n-Hexane using in-vitro method.
**Method:** The experimental study was carried out at University of Karachi and Pharmacology Department of Baqai Medical University, using in-vitro methods for a period of six months from November 2023 to May 2024.The antioxidant property of leaf extracts of *D. regia* in polar and non-polar solvent i.e. acetone and hexane respectively, was evaluated by using DPPH radical scavenging assay. Gallic acid serves as the reference compound. Statistical analysis was based on Mean ± SEM because data was obtained in technical triplicates. The $IC_{50}$ (Inhibitory concentration 50) was calculated from dose response curve.
**Results:** Acetone extract of *D. regia* leaves exhibited 89.9% inhibition at 0.5 mg/mL concentration and had $IC_{50}$ of 133.5 µg/mL, indicating significant antioxidant property. However, no activity was detected in the hexane extract of *D. regia* leaves. At the concentration of 0.5 mg/mL, its antioxidant potential is unclear. Comparatively, Gallic acid exhibited strong antioxidant activity with 95.3% inhibition at 0.5 mg/mL.
**Conclusion:** In conclusion, this study showed that leaf extracts of *D. regia* in polar solvent (acetone) exhibited significant antioxidant potential. This emphasizes that leaves extract of D. *regia* is a promising source of antioxidants.
**Keywords:***Delonix regia*, Antioxidant, Acetone, n-Hexane, DPPH, Gallic Acid**.**

**INTRODUCTION:** Currently, there is a prominent surge to develop interest towards natural drugs and excipients. With the steady decline in overall health, individuals are increasingly looking for antioxidants from natural sources and searching healthier option. Nutraceuticals have appeared as an important market segment, gather attention for their potential health benefits. Food fortification is a feasible approach that involves enriching deficient food products with essential nutrients, such as vitamins and minerals, either from natural sources or through artificial synthesis. *D. regia* stands out as a natural specie plentiful in antioxidants and carotenoids. This flowering plant is with many names, for example flamboyant, Peacock, flame tree, Gulmohar, and Royal Poinciana, is widely present in regions including Mexico, Australia, India, the Caribbean, Northern Mariana Islands, South Floridaand, UAE [1,2,3].

Significantly investigated, *D. regia* exhibits various biological activities, including antioxidant [4], anti-arthritic, antidiarrheal [5], hepato-protective, cytotoxic [1], gastro-protective [6], antimicrobial [4, 7], anthelmintic, anti-cancer, anti-rheumatic, anti-malarial, anti-diabetic properties [8], and pest control [9]. Rich in a spectrum of pigments including anthocyanins, flavonol glycosides [3,10], carotenoids, phenolic acids [11], tannins, alkaloids, saponins, steroids, and β-sitosterol [12]. *D. regia* leaf extracts have showed remarkable anti-inflammatory and anti-oxidant properties. The buildup of reactive oxygen species (ROS) in cells and tissues results in oxidative

stress, which poses substantial harm to the human body. This process is implicated in the development of numerous chronic conditions such as diabetes, cancer, and metabolic disorders. On the other hand, the detrimental effects of oxidative stress can be alleviated through the intervention of antioxidant compounds. These compounds have the capacity to diminish or prevent cellular damage caused by free radicals. As a result, the examination and application of antioxidants offer promising strategies for mitigating the progression and severity of oxidative stress-related diseases, thereby enhancing health outcomes [5].

The abundance of antioxidants emphasizes the various applications of natural ingredients across different products. *D. regia* emerges as a vital source of antioxidants, helping to mitigate the formation of ROS within the body. In present study, we evaluated the antioxidant potential of *D. regia* leaf extracts to explore the potential healthcare benefits of this plant.

**METHOD:** The study was carried out experimentally at University of Karachi (UoK) and Pharmacology Department of Baqai Medical University, using in-vitro approaches for a period of six months from November 2023 to May 2024. This study was permitted by the Institutional Review and Ethical Board (IREB) of Baqai Medical University, Ref. No. BMU-IREB-03-2023 dated 02-08-2023. The leaves of *D. regia* were acquired from the garden of UoK. Subsequently, identification and authentication of leaves of *D. regia* was performed at the herbarium of Department of Botany, UoK, receiving the voucher no. 97626.

The freshly picked leaves of *D. regia* were collected and carefully separated. They underwent a thorough cleaning procedure with tap water for removing any attached dirt, followed by rinsing with water to remove impurities. Afterward, these were dried in air, then chopping was carried out, and at last leaves were grinded in the blender. The extraction was performed on final powdered product using different solvents (acetone and hexane) in a Soxhlet apparatus. Rotary vacuum evaporator was used to concentrate the resulting solutions and then it was stored in a desiccator to maintain its integrity and prolong its shelf life, ensuring its suitability for further analysis and potential applications [13,14,15,16].

DPPH Radical Scavenging Assay: DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) is a dark-color crystalline powder comprising of stable free-radicals. It possesses wide application in laboratory research, especially in antioxidant assay. In its radical form, DPPH dissolved in ethanol appears deep violet and exhibits greater absorbance at 517 nm. Upon reaction of DPPH with antioxidant like vitamin C, it is reduced to its molecular form (DPPHH), causing a change in colour to pale yellow and a decline in absorbance. This change in absorbance is used to assess the radical scavenging ability of the tested samples.

Pure sample: 0.5 mM in DMSO
Crude Sample: 0.5 mg/mL in DMSO
DPPH solution: 0.3 mM in Ethanol

Solution of DPPH (95µl, 300µM) in ethanol was combined with a test solution (5µl, 500µM). This mixture was kept at 37ºC for half hour to allow the reaction then the absorbance was measured using a Spectra Max340 multiplate reader at 517 nm. As the reaction progresses, the color of the solution changed from violet to pale yellow, indicating reduction. The percentage of radical scavenging activity (%RSA) was determined by comparing the results with a control having DMSO. The $IC_{50}$ value was the concentration that reduced initial DPPH concentration by 50%. EZ-Fit Enzyme kinetics software program (Perrella Scientific Inc. Amherst, MA, USA) was used for determining $IC_{50}$ values of compounds. Gallic acid was used as the reference standard [17].

**RESULTS:** The results of DPPH scavenging activity of acetone and n-hexane extracts, respectively are represented in Table 1. The results of the antioxidant properties estimation for numerous extracts derived from *D. regia* provide insight into their efficacy.

**Table 1**: Antioxidant Property of Various Extracts of *Delonix regia* leaves comparison with Gallic acid (control)

| Sample Code | Concentration (mg/mL) | % Inhibition | IC50 ± SEM |
|---|---|---|---|
| Acetone extract of *Delonix regia* leaves | 0.5 | 89.9 | 133.5±0.80 µg/mL |
| n-Hexane extract of *Delonix regia* leaves | 0.5 | -3.3 | - |
| Gallic Acid (STD) | 0.5 | 95.3 | 3.69±0.04 µg/mL |

**DISCUSSION:** Reactive oxygen species, which encompass the superoxide and hydroxyl radicals, peroxynitryl, and hydrogen peroxide, undergo continuous synthesis within living systems due to metabolic reactions [18].These ROS are known for their role in contributing to various diseases linked to oxidative stress. As a result, it becomes crucial to detoxify or scavenge such ROS utilizing natural products to strengthen the host defense system against oxidative damage and associated ailments. Previous research, such as that by Chhabra and Gupta, reported that acetone and water extracts of flower petals of *D. regia* showed more than 90% DPPH scavenging property, and water extract has more antioxidant effect[19]. Vivek et al., (2013) reported that methanolic extract of leaves and flower of *D. regia* had antioxidant properties[7]. Study by Shabir et al., showed that leaves extract of *D. regia* in absolute acetone and 80% acetone exhibited moderate antioxidant activity 48% and 52% respectively [20]. Khan et al., (2020) also reported that methanolic aerial part extract of *D. regia* had hypoglycemic, antioxidant, and hypolipidemic activities [21]. We determined the antioxidant activity of leave extract in acetone and n-hexane and found that acetone extract had 89.9% radical scavenging activity, however, n-hexane extract showed no activity. In our previous study, we identified the presence of phytochemicals such as Vitamin E, α-Amyrin, Lupeol, and Squalene in the acetone extract [22], which contributed to its strong antioxidant activity observed in current study. Although the acetone extract of *D. regia* shows promising results, the n-Hexane extract seems to lack significant antioxidant potential. The data suggests that extract of polar solvent (acetone extract) has antioxidant activity which may be due to water or polar solvent soluble constituents like phenolic acid. This may be the reason of better antioxidant potential of acetone extract. Future studies should investigate the specific antioxidant compounds present in the extract of *D. regia* in different types of solvents and its potency should be optimized. Moreover its potential therapeutic applications in managing oxidative stress and formulation of pharmaceutical and nutraceutical products should be explored.

**CONCLUSION:** In conclusion, our assessment of the antioxidant activity of extracts of *Delonix regia leaf* in acetone and hexane revealed distinct outcomes. The acetone extract of *D. regia* leaves demonstrated a significant 89.9% inhibitory activity at a concentration of 0.5 mg/mL, indicating a substantial level of antioxidant property. This finding highlights the potential of above-mentioned extract as a source of antioxidants.

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