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In-Vitro Analysis of the Anti-Diabetic Potential of Acetone and n-Hexane Extracts of Delonix regia (Gul Mohar) Leaves

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Author`s Contribution

All authors contributed equally to this research work and paper write-up.

Funding Source: None
Conflict of Interest: None

Received: Aug 25, 2024
Accepted: Jan 11, 2025

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ABSTRACT

Objective: To assess the anti-diabetic activity of leaves extract of D. regia in acetone and n-hexane through in vitro analysis.

Methodology: The preclinical study was conducted in the Department of Pharmacology and Therapeutics at Baqai Medical College/University of Karachi, in November 2022. Freshly harvested D. regia leaves were collected identified, and authenticated by the herbarium of the botany department at Karachi University. The extract was concentrated in rotary vacuum evaporator and kept in a desiccator to maintain their integrity and suitability for further applications. In vitro, anti-hyperglycemic studies were conducted using α-amylase and α-glucosidase inhibition assays.

Results: D. regia leaves extract revealed distinct inhibitory activity of α-amylase and α-glucosidase. Acetone extract (25 mg/ml) showed 7.8% and 2.5% inhibition, respectively; while the n-hexane extract (25 mg/ml) demonstrated lower activities, 1.4% and 2.0%, respectively.

Conclusion: The present study concludes that polar solvent (acetone) extract of D. regia exhibits more inhibitory potential for α-amylase and α-glucosidase as compares to non-polar solvent emphasizing solvent polarity's role in extracting active compounds.

Keywords: Delonix regia, α-amylase, α-glucosidase, acetone, n-hexane, anti-hyperglycemia.

Cite this article as: Sheikh SA, Ahmed A, Rasheed S, Waqar SH, Asadullah, Haque SU, Nawaz S. In-Vitro Analysis of the Anti-Diabetic Potential of Acetone and n-Hexane Extracts of Delonix regia (Gul Mohar) Leaves. Ann Pak Inst Med Sci. 2025; 21(1):136-140. doi. 10.48036/apims.v21i1.1481.

Introduction

Diabetes mellitus (DM) is a chronic disorder associated with persistent elevated blood glucose levels due to hormonal imbalances. This condition arises from a decrease in insulin production by the β cells of the pancreas, which impairs cellular glucose metabolism, leading to elevated blood glucose levels and hyperglycemia.[1] Hyperglycemia increases the production of free radicals within cells, which in turn contributes to oxidative stress, and further damage pancreatic β cells.[2] The International Diabetes Federation (IDF) estimates that in 2021, approximately 536.6 million individuals having DM, whether identified or not identified. By the year 2045, this number is expected to rise by 46%, which is 783.2 million.[3]

The standard treatment for DM includes oral medications and insulin injections. However, these treatments cause side effects that include hypoglycemia, headache, dizziness, diarrhea, nausea, bloating, flatulence, loss of appetite, liver toxicity, weight gain, abdominal swelling, vitamin B12 deficiency, lactic acidosis, and a heightened risk of cardiovascular diseases.[4] Due to the risks linked to these side effects, this study focuses on exploring

medicinal plants as safer alternatives for treating diseases like diabetes.[5]

One medicinal plant with recognized anti-diabetic properties is the flamboyant flower (Delonix regia).[6] Commonly known as the flame tree, D. regia is an ornamental species belonging to the legume family. The Delonix genus consists of two species: D. regia and D. elata. D. regia produces distinctive five-petaled flowers, with four petals sharing the same color while the fifth petal is uniquely marked with white streaks.[7]

This plant is rich in bioactive compounds, including flavonoids, phenolic compounds[8], carotenoids, and anthocyanins.[9] Flavonoids and phenolic compounds are widely recognized for their anti-diabetic effects [10], while carotenoids and anthocyanins serve as potent antioxidants.[10] These bioactive compounds have been linked to improved heart function[11], reduced blood glucose levels[12, 13], and the potential to restore pancreatic β-cell function in individuals with diabetes mellitus.[14] However, research on the anti-diabetic properties of Delonix regia remains limited, necessitating further investigation.

Numerous studies have investigated various plant parts rich in bioactive compounds.[15-17] The promising findings from these studies have stimulated further research. Consequently, we have selected *D. regia* leaves for examining their anti-diabetic properties, using both polar (acetone) and non-polar (n-Hexane) solvents for extract preparation.

Methodology

The preclinical study was conducted in the Department of Pharmacology and Therapeutics at Baqai Medical College/University of Karachi, using in-vitro methods for 6 months.

In November 2022, D. regia leaves were sourced from the Karachi University garden and authenticated at the Botany Department's herbarium (voucher number 97626).

Freshly harvested D. regia leaves were collected, cleaned thoroughly with tap water to remove dirt, and rinsed with distilled water to eliminate contaminants. After air-drying, the leaves were chopped, blended into a fine powder, and subjected to extraction using n-Hexane and Acetone solvents in a Soxhlet apparatus. The resulting extract was concentrated via rotary vacuum evaporator and kept in a desiccator to maintain their integrity and suitability for further analysis and potential applications.[18-21]

Alpha-glucosidase (0.2 units/ml from *Saccharomyces cerevisiae*) was mixed in 0.05M buffer of potassium phosphate (pH: 7). The substrate, p-nitrophenyl-alpha-D-glucopyranoside, was also dissolved in the same buffer at a concentration of 5 mmol/L. The mixture of reaction contained 0.5 ml of the buffer, 0.1 mL enzyme, and 25 mg/ml of extract solution in hexane was kept for incubation for 20 minutes at 37°C. After adding and mixing 0.5 ml of the substrate, the mixture was incubated for twenty minutes at 37°C. Then 1.0 mL of 20% sodium carbonate was added to stop the reaction. Absorbance was determined at 420 nm for measuring enzymatic activity. Control samples contained acarbose as a standard instead of leave extracts with similar concentration. The α-glucosidase inhibitory potential was expressed as a percent inhibition.[22]

$$\text{Percent inhibition (\%)} = A_{\text{control}} - A_{\text{Sample}} / A_{\text{Control}} \times 100$$

Where 'A' is Absorbance

Reaction mixture, which was comprised of 0.5 ml of potassium phosphate buffer (0.05 M; pH 7.0), 0.1 ml of alpha-amylase enzyme from *Bacillus subtilis* (10 mg/ml), and plant extracts at a conc. of 25 mg/ml, was kept at 37°C for pre-incubation of 20 minutes. Next, one mL of 1% soluble starch solution which was prepared in 0.05 M potassium phosphate buffer of pH 7.0 was added and it was subsequently incubated for another 20 minutes at 37°C. Afterward, 1.0 ml of DNS reagent was added to terminate the reaction followed by heating in water bath for 5 min. After cooling, solution was diluted by adding 9.0 ml of distilled water, and then absorbance was measured at 540 nm. Preparation of Control was done without plant extract using acarbose as a standard with similar concentration.

The inhibitory potential of α-amylase was expressed as percent inhibition.[23]

$$\text{Percent inhibition (\%)} = A_{\text{control}} - A_{\text{Sample}} / A_{\text{Control}} \times 100$$

DNS reagent was formulate by mixing 1.0 g of 3,5-dinitrosalicylic acid in 50 ml of deionized water. Next, potassium sodium tartrate (tetra-hydrate) and 2N sodium hydroxide, 30.0 g and 20.0 mL respectively were added. Dilution of mixture was performed to a final volume of 100 ml with deionized water. Then this solution was kept at 4°C in brown reagent bottle up to 2 weeks.

Stock standard (5.0 mg/ml) of Acarbose (Glucobay) was prepared in DMSO. Then dilution of 250 µg/ml was made by 0.1 M buffer of potassium phosphate (pH 7.0).

Results

Assays of α-amylase and α-glucosidase were used to monitor inhibitory activity of *D. regia* leaves extract. It possessed distinctive inhibitory activities of α-amylase and α-glucosidase when extracted via acetone and n-hexane solvents. Extract of acetone (25 mg/ml) exhibited an alpha-amylase inhibition of 7.8% and an alpha-glucosidase inhibition of 2.5%. (Table I).

Table I: Inhibitory activity of leave extract of *D. regia* in acetone.

Sample Description	Parameter	% inhibition
Leave extract of *D. regia* in Acetone (25 mg/ml)	α-amylase inhibition	7.8%
	α-glucosidase inhibition	2.5%

Despite the fact, leave extract of n-hexane (25 mg/ml) of *D. regia*, also demonstrated considerably lower inhibitory activities. The α-amylase inhibition was 1.4%, and α-glucosidase inhibition was 2% (Table II). These results suggest that the n-hexane extract is less effective in inhibiting these enzymes compared to the acetone extract (Table I and II).

Table II: Inhibitory activity of leave extract of *D. regia* in n-Hexane.

Sample Description	Parameter	% inhibition
Leave extract of *D. regia* in n-Hexane (25 mg/ml)	α-amylase inhibition	1.4%
	α-glucosidase inhibition	2.0%

Discussion

DM, a metabolic condition, is associated with persistent increased blood glucose levels, affecting the metabolism of carbohydrates, lipids, and proteins to varying degrees.[24] Medically, it encompasses a range of metabolic conditions linked to hyperglycemia caused by partial or total insulin insufficiency. DM imposes economic burdens due to the high costs of treatment and associated premature morbidity and mortality. For individual patients, it is a lifelong condition requiring careful management of diet, lifestyle, and blood glucose levels, along with regular medication.[25] Many treatments involving medicinal plants are recommended, as most plants with anti-diabetic effects contain compounds like carotenoids, glycosides, terpenoids, flavonoids and alkaloids. These plants often exert anti-hyperglycemic effects by enhancing activity of pancreatic tissues, either by increasing insulin secretion or reducing glucose absorption in the intestine.[26] Literature reviews have shown that various parts of *D. regia* have anti-diabetic properties, prompting this study to investigate the anti-hyperglycemic activity of its leaves.

The research result suggested the differential inhibitory effects of *D. regia* leaf extracts on α-amylase and α-glucosidase enzymes, dependent upon the solvent utilized for extraction. The acetone extract produced 7.8% and 2.5% inhibition of α-amylase and α-glucosidase respectively. This proposes that the acetone extract contained constituents with a moderate capability to hinder the activity of these carbohydrate hydrolyzing enzymes, which are significant in the regulation of post-prandial glucose levels in blood.

However, the n-hexane extract exhibited lower inhibitory activities, with 1.4% α-amylase inhibition and 2% inhibition of α–glucosidase. Yadao et al. reported the in vitro α-glucosidase inhibitory action of extracts of *D. regia* in chloroform, methanol, petroleum ether, ethyl acetate, and aqueous. Along with all the extracts, methanol extracts of *D. regia* leaves demonstrated the most promising activity having IC50 of 83.46 µg/ml and 75% inhibition.[27] In-vitro anti-hyperglycemic study by Abarnadevika et al, showed that hydro-alcoholic extract of *D. regia* bark exhibited IC_{50} of 167 µg/mL and 116.31 µg/mL in α -amylase and α-glucosidase inhibitory assay, respectively.[28] In his study. Another study showed that methanolic extract of leaf of *D. regia* produced anti-hyperglycemic effects (42.46%) at 400 mg/kg dose in hyperglycemic mice.[29] Similarly, research by Chaturvedi et al. also reported the anti-diabetic effect of an aqueous suspension of flamboyant flower leaf extracts (100 and 200 mg/kg) in alloxan-induced rat model of hyperglycemia.[30] However, in our study, the lower efficacy exhibited by extract of n-hexane indicates that the active components responsible for enzyme inhibition are extra soluble in polar solvents similar to acetone than in non-polar solvents similar to n-hexane. The inhibitory activities showed with the acetone extract perhaps due to the presence of certain phyto-chemicals, for example flavonoids and phenolic acids, which are known for their enzyme inhibition properties and are more readily extracted with polar solvents. This variation highlights the crucial role of solvent polarity in efficiently extracting bioactive compounds from plant materials.

Based on these findings, further research is needed to investigate different solvent systems for extraction and

evaluate their impact on bioactive compound recovery. Future studies should focus on isolating and characterizing the specific bioactive compounds present in *D. regia* leaf extract prepared using acetone. Understanding the mechanisms of action of these compounds will provide deeper insights into their inhibitory effects on α-amylase and α-glucosidase. Additionally, in vivo studies are essential to confirm the efficacy and safety of these extracts in managing postprandial hyperglycemia. Exploring various extraction techniques and solvents may help optimize the yield of active compounds. Finally, assessing the long-term therapeutic potential and any possible adverse effects of these extracts is crucial for their development as natural anti-diabetic agents.

Conclusion

This study concludes that the polar solvent (acetone) extract of *D. regia* demonstrates greater inhibitory activity against α-amylase and α-glucosidase compared to the non-polar solvent extract, suggesting its potential for managing postprandial hyperglycemia. These findings emphasize the importance of selecting appropriate solvents to effectively extract bioactive compounds and maximize the therapeutic potential of *D. regia*.

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