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Evaluation Of The Invitro Antidiabetic Activity of *Delonix Regia* Flower Extract

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ABSTRACT :

Delonix regia, commonly known as the flamboyant tree, has been traditionally used in various cultures for its medicinal properties. Recent studies suggest its potential in managing diabetes, a condition characterized by elevated blood glucose levels. This research investigates the Invitro antidiabetic effects of Delonix regia flowers extract.

Keywords: Diabetes Mellitus, Medicinal Properties, elevated blood glucose level,Invitro

Diabetes mellitus is a serious, complex chronic condition that is a major source of ill health all over the world. This metabolic disorder affects approximately 4% of the population worldwide and is expected to increase to 5.4% in 2025. Diabetes mellitus is characterized by hyperglycemia and carbohydrate, protein, and fat metabolism disturbances.[1] Increased thirst, increased urinary output, ketonemia and ketonuria are the common symptoms of diabetes mellitus, which occur due to the abnormalities in carbohydrate, fat, and protein metabolism.[2]

There are three types of diabetes: diabetes type I, diabetes type II and gestational diabetes. In the case of type I diabetes, the pancreas produces very little insulin or sometimes no insulin at all. Type I diabetes attacks the pancreatic cells and stops their functioning. Around 5 to 10% of all diabetes is type I and it occurs not only in childhood or adolescence, but also in adulthood. Type diabetes II occurs if the body does not produce insulin properly. For this reason, glucose metabolism is affected and the body cannot convert the glucose into energy. Around 90% of all diabetes in the world is type II diabetes. Adults as well as children can be affected by type II diabetes. The third type of diabetes, gestational diabetes mellitus (GDM), resembles type II DM in several respects, as it involves a combination of comparatively inadequate insulin secretion and responsiveness. About 2-10% of all pregnant women are affected by gestational diabetes, which may progress or vanish after delivery.[3]

The common strategy for treatment focused mainly on regulating and decreasing blood sugar to fall within the normal level. The main mechanisms in both traditional and Western medicines involve decrease blood sugar through stimulating pancreatic β- cells; inhibiting other hormones elevating blood sugar; increasing the affinity, and sensitivity of insulin receptor. On the other hand, lowering glycogen release; enhancing glucose utilization within many tissues and organs; clearing free radicals, resisting lipid peroxidation, correction of the lipid and protein metabolic disorders and improving human blood circulation are also involved. The present oral antidiabetics include sulfonyl urea that decrease blood sugar, mainly by elevating insulin release from islets of Langerhans. They combine with sulfonylurea receptor on β-cells resulting in adenosine triphosphate–dependent potassium channels closure. Consequently, the cell membrane depolarizes and its resistance. Traditionally, it is predominant among elderly people (over 40 years). Commonly, it occurs in people with obesity, decreased body activity, and it may also be inherited. The disease is often enhanced through dietary supplements, physical activity along with oral hypoglycemic agents. Moreover, there is another temporary disease related to diabetes, which is known as gestational diabetes (GD) mellitus. It refers to the occurrence or initial recognition of glucose intolerance during the period of pregnancy. Other types of diabetes include genetic abnormalities in the ß-cell of the pancreas or mutations in insulin receptor or post-receptor deformities. Diseases of the exocrine pancreas such as pancreatitis, cystic fibrosis and excessive production of insulin counter regulatory hormones such as Cushing's syndrome and acromegaly ultimately leads to Diabetes mellitus. Many drugs exemplified by glucocorticoids, niacin and interferon may induce Diabetes mellitus.[4]

Herbal medicines have been used for thousands of years in many ethnic cultures such as Chinese, Korean, Indian and Mexican to treat and manage diabetes and its complications. In the last few decades, modern science has uncovered the benefits of using herbal medicines in the management of particular diabetic complications, such as vascular inflammation, nephropathy and retinopathy.[5]

Herbal drugs have advantage of being easily available, less side effects and low cost, because of these advantages the search for traditional drug have become more. The modern society is running towards the herbal drugs. The relevance of any product of herbal origin should have essential marketing strategy. Because these days the people think that if it is herbal origin then it's safe. From the ancient time plants have always been rich source of drugs. A number of drugs have derived directly and indirectly from plants.[6]

Traditional medicine, as defined by the World Health Organization, is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. Some traditional medicine systems are supported by huge volumes of literature and records of the theoretical concepts and practical skills; others pass down from generation to generation through verbal teaching. To date, in some parts of the world, the majority of the population continue to rely on their own traditional medicine to meet their primary health care needs. When adopted outside of its traditional culture, traditional medicine is often referred as "complementary and alternative medicine." Among others, the most widely used traditional medicine systems today include those of China, India, and Africa.[7]

Delonix regia (Bojer ex Hook) Raffin (Poinciana regia, Royal Poinciana, Gul mohar, Flame tree or Flamboyant; Fabaceae-Caesalpinioideae) is a large ornamental tree with fern-like bipinnately compound leaves and attractive red peacock flowers and native to Madagascar. The flowers, leaves and barks contain most of the active constituents. The flowers possess insecticidal, antifertility, wound healing, antifeedant, anthelmintic activities and also inhibit the malaria parasite in humans.[8]

The common constituents present in flowers of the plant also contain flavanols such as quercetin and its glycosides (quercetin3-O-glucoside, quercetin-3-O-galactoside, rutin, quercetin3-O-robinobioside, and quercetin triperoxide), as well as kaempferol rhamnosylhexoside and isorhamnetin rhamnosylhexoside.[9]

Current allopathic treatment for the diabetes is associated with the side effect and adverse effect. So, there is need to search for the safe herbal sources which can resolve the problem of diabetes therefore in consideration of above facts the present study is aimed at evaluating the Invitro anti-diabetic potential of ethanolic extract of Delonix regia flowers .

MATERIALS AND METHOD

Flower of Delonix regia were collected from local areas of JMIT campus, Chitradurga, Karnataka and they were washed, then the flower were dried in fresh circulating air under shade for seven days. The flower material was identified and authenticated by Miss. Niveditha B T, Assistant professor, Dept of studies in Botany, Jyanagangothri PG Center, GR Halli, S Davanagere university, Chitradurga, Karnataka.

METHOD OF PREPARATION [10]

Delonix regia flower were collected from local areas of JMIT campus Chitradurga.The flower were dried in fresh circulating air under shade for seven days, flower were subjected to size reduction by dry grinder.Dried flower powder was subjected to Soxhlet apparatus and extracted with ethanol (70% v/v) at 40°C. For each gram of powder 2 ml of solvent was used.The extract was filtered through Whatman No.1 filter paper.The residue obtained was designate as crude extract and stored in a freezer.The stock solutions of Ethanolic extracts were prepared using Distilled water and ethanol which is used for following studies:Study of Preliminary phytochemical investigation.

Preliminary phytochemical investigation[11,12]

The extracts obtained were subjected to the preliminary qualitative phytochemical investigation and subjected to the following qualitative tests to detect the presence of chemical constituents.

Sl. No	Phytoconstituents	Ethanolic Extract
1.	Tannins	+
2.	Saponins	++
3.	Triterpenoids	_
4.	Flavonoids	+
5.	Resins	+
6.	Glycosides	+
7.	Alkaloids	++
8.	Steroids	+
9.	Carbohydrates	+++
10.	Phenols	+

+Present,-Absent,++Higher concentration.

PROCEDURE

a-amylase inhibition activity[13]

Procedure:

The plant extract of different concentration such as 50, 100, 250, 500 and 1000µg were taken and dissolved with 0.25ml of α- amylase solution and mixed thoroughly. The sample was incubated at 37°c for 5minutes. Add 5ml of starch solution and incubate for 3 minutes at 37°c.Then 3ml of DNSA reagent was added and boiled at 100°c for 5minutes to stop the reaction. The reaction mixture was cooled to room temperature and the absorbance was read at 540nm in spectrophotometer. The α- amylase inhibition activity was calculated using the formula:

$$\% \, Inhibition = \frac{\text{Absorbance 1} - \text{Absorbance2}}{\text{Absorbance 1}} \times 100$$

Where Absorbance 1- control , Absorbance 2- standard

A. Glucosidase inhibitory assay.[14]

Procedure:

0.2ml of α- glucosidase enzyme solution was prepared and preincubated with different concentrations of the test and standard drug solution for 5 minutes. To all the test tube 0.2ml of 37Mm sucrose are added. All the tubes were incubated for 30 minutes at 37°c. to allow the enzymatic action and drug action. After 30min, the tubes are taken out from the incubator and heated at 100°c for 10 minutes. The liberated glucose is determined by glucose oxidase peroxidase method at 546nm and calculating with relative blank control.

The α- glucosidase inhibition activity was calculated using the formula:

$$\%Inhibition = \frac{\text{Absorbance1} - \text{Absorbance 2}}{\text{Absorbance 1}} \times 100$$

Where Absorbance 1- control , Absorbance 2- standard

Absorbance 2 – standard/test

B. Starch Iodine Assay:[15]

Procedure:

Approximately 1 mL of plant extract of different concentration (0.1–10 mg/mL) was taken in test tubes. A volume of 20 µL of α-amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation, 200 µL of 1% starch solution was added to each test tube and the mixture was further incubated for 1 h at 37 °C. Then, 8 mL of distilled water was added. Absorbance of the mixture was taken at 565 nm. Each experiment was done in triplicate. The α-amylase inhibitory activities were calculated as percentage inhibition. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC50) were determined graphically.

RESULTS AND DISCUSSION

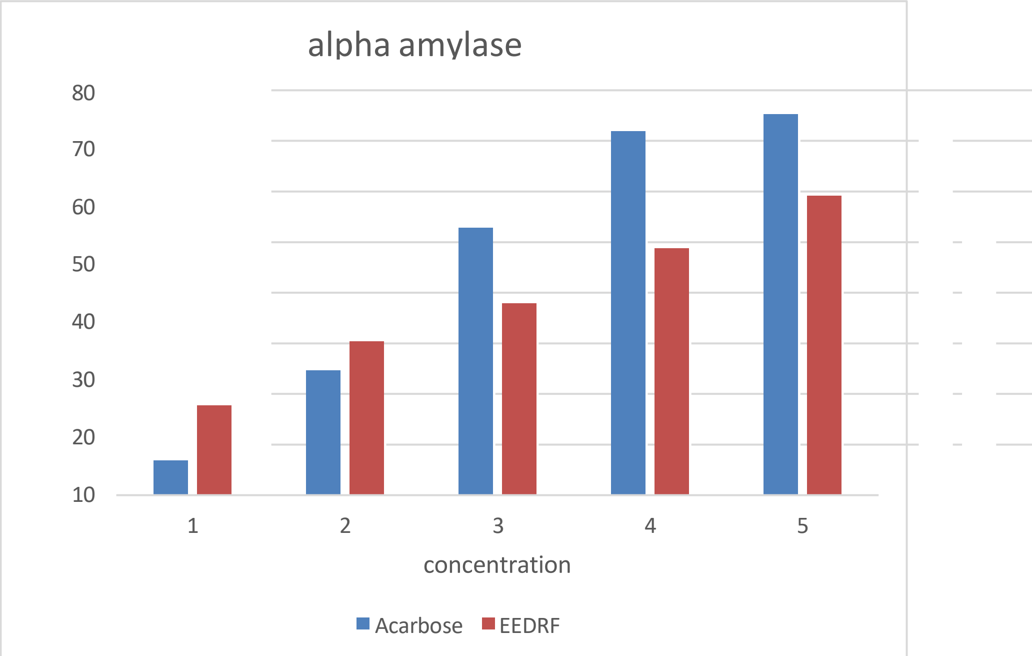
1. Assay for α-amylase Inhibitory Activity:

The results of in-vitro antidiabetic activity using a-amylase inhibitory assay of the Ethanolic Extract of flowers of Delonix regia. The percentage inhibition at 20-100µg/ml concentrations of EEDRF showed a dose dependent increase in percentage inhibition. The percentage inhibition of EEDRF varied and have shown from 4.02% to 46.55% with an IC50 value of 93.06 µg/ml and Acarbose is a standard drug for a-amylase inhibitor. Acarbose at a concentration of (20-100µg/ml) showed have shown from 6.89 to 75.28% with an IC50 value of 48.13 µg/ml.

In-vitro Anti diabetic activity of Ethanolic Extract of flowers of Delonix regia, Using α-amylase Inhibitory Activity and comparison with Acarbose.

Sl.No	Concentration (µg/ml)	Acarbose		EEDRF	
		Mean	% of inhibition	Mean	% of Inhibition
1.	20	1.62	6.89 %	1.43	17.81%
2.	40	1.31	24.71 %	1.21	30.45%
3.	60	0.82	52.87 %	1.08	37.93%

4.	80	0.51	71.92 %	0.89	48.85%
5.	100	0.43	75.28 %	0.71	59.19%
	IC50	**48.13 µg/ml**		**95.48 µg/ml**	



Effect of EEDRF α-Amylase assay

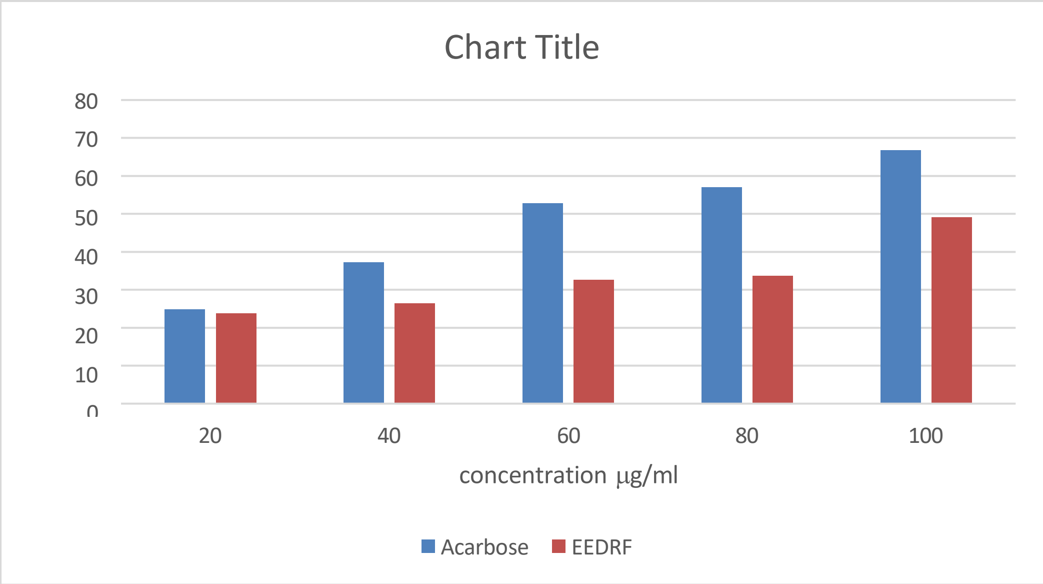
1. ***In-vitro* Anti diabetic activity of Ethanolic Extract of flowers of *Delonix regia*,**

Using α-glucosidase Inhibitory Activity and comparison with Acarbose.

The results of *in-vitro* antidiabetic activity using α-glucosidase inhibitory assay of the Ethanolic Extract of flowers of *Delonix regia* shown in **Table 7 and Figure 6.** The percentage inhibition at 20-100µg/ml concentration of EEDRF shown a dose dependent increase in percentage inhibition. The percentage inhibition of EEDRF varied and have shown from 23.83% to 49.22% with an IC50 value of 97.67µg/ml and Acarbose is a standard drug for α- glucosidase inhibitor. Acarbose at a concentration of (20-100µg/ml) have shown from 24.87 to 66.83% with an IC50 value of 51.23 µg/ml.

***In-vitro* Anti diabetic activity of Ethanolic Extract of flowers of *Delonix regia*, Using: α-glucosidase Inhibitory Activity and comparison with Acarbose.**

Sl.No	**Concentration (µg/ml)**	**Acarbose**		**EEDRF**	
		Mean	**% of inhibition**	**Mean**	**% of Inhibition**
1.	20	1.45	24.87 %	1.47	23.83%
2.	40	1.21	37.30 %	1.42	26.42%
3.	60	0.91	52.84 %	1.30	32.64%
4.	80	0.83	56.99 %	1.28	33.67%
5.	100	0.64	66.83 %	0.98	49.22%
	IC50	**51.23 µg/ml**		**97.67 µg/ml**	



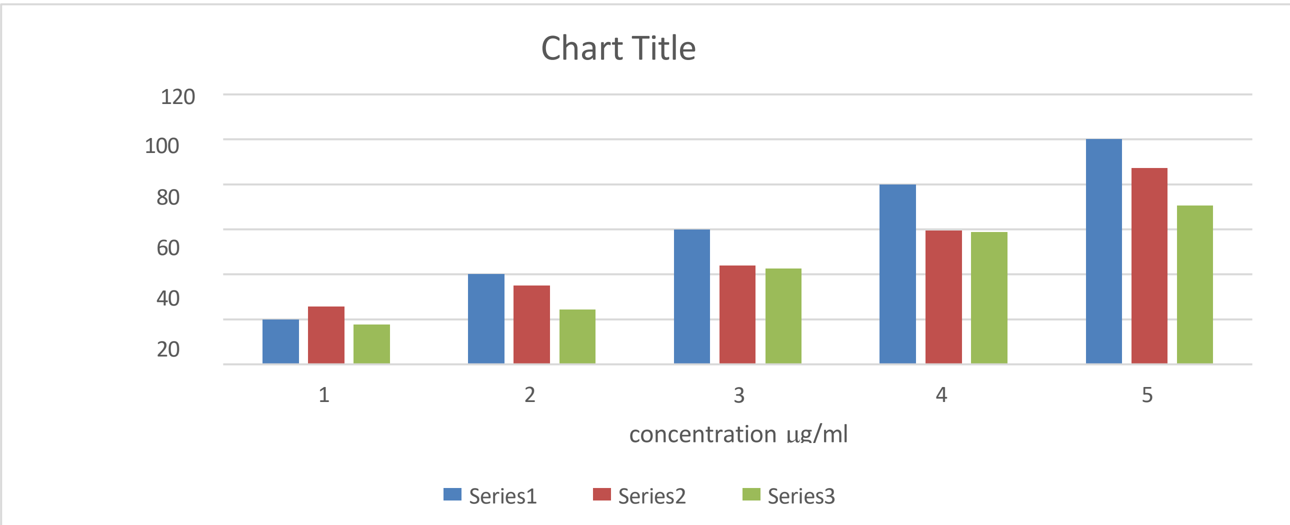
Effect of EEDRF α-glucosidase assay

1. ***In-vitro* Anti diabetic activity of Ethanolic Extract of flowers of *Delonix regia*, Using: starch iodine assay and comparison with Acarbose.**

The results of *in-vitro* antidiabetic activity using starch iodine assay of the Ethanolic Extract of leaves of *Delonix regia* shown in **Table 8 and Figure 7.** The percentage of starch iodine decolourisation shown at 20-100µg/ml concentration of EEDRF shown a dose dependent increase in percentage decolourisation. The percentage decolourisation of EEDRF varied and have shown from 17.78% to 70.44% and Acarbose is a standard drug for α- glucosidase inhibitor. Acarbose at a concentration of (20-100µg/ml) have shown from 25.64% to 87.24% of decolourisation.

***In-vitro* Anti diabetic activity of EEDRF Using: Starch Iodine decolourisation Activity and comparison with Acarbose**

Sl.No	Concentration (µg/ml)	Acarbose		EEDRF	
		Mean	% of decolourisation	Mean	% of decolourisation
1.	20	1.102	25.64%	1.217	17.78%
2.	40	0.962	35.08%	1.121	24.35%
3.	60	0.832	43.85%	0.835	42.65%
4.	80	0.603	59.31%	0.611	58.77%
5.	100	0.189	87.24%	0.438	70.44%



Effect of EEDRF starch iodine assay

DISCUSSION

This study aimed to evaluate the anti-diabetic properties of *Delonix regia* flowers through in vitro approaches, The results demonstrate significant therapeutic potential for *Delonix regia*, impacting various metabolic and biochemical parameters.The in vitro assays revealed that *Delonix regia* flower extract effectively inhibits alpha-amylase and alpha-glucosidase activities. These enzymes are crucial in carbohydrate digestion; alpha-amylase catalyzes the breakdown of starch into simpler sugars, while alpha-glucosidase further breaks down these sugars into glucose. By inhibiting these enzymes, the extract may delay glucose absorption in the intestines, leading to lower postprandial blood glucose levels. This mechanism is vital for managing diabetes, as it can help prevent sharp spikes in blood sugar after meals. starch-iodine assay complemented these findings by demonstrating that the extract reduces starch digestibility. The decrease in the ability to hydrolyze starch into glucose further supports the notion that *Delonix regia* can modulate carbohydrate metabolism effectively. These results are consistent with prior studies highlighting the role of natural compounds in managing diabetes through enzyme inhibition.

CONCLUSION

Diabetes is a chronic condition characterized by high blood sugar levels due to issues with insulin production or function. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these harmful compounds or repair the resulting damage. Factors such as hyperglycemia, inflammation, and environmental stressors can increase ROS levels, leading to cellular damage, lipid peroxidation, and DNA mutations. In the context of diabetes, oxidative stress plays a significant role in the development and progression of the disease. Elevated blood glucose levels can enhance the production of ROS, contributing to complications such as neuropathy, nephropathy, and retinopathy. Cellular Damage: Oxidative stress damages pancreatic beta cells, impairing insulin secretion. Antioxidants can help protect these cells, promoting better insulin release and improving overall glycaemic control.Insulin Sensitivity: Chronic oxidative stress is linked to insulin resistance. Antioxidants may enhance insulin sensitivity by reducing oxidative damage to insulin receptors and signalling pathways.The present study investigated the potential anti-diabetic properties of *Delonix regia* flower extract through a comprehensive in vitro screening approach,The findings from the in vitro assays including the alpha-amylase and alpha- glucosidase inhibition assays, along with the starch-iodine test indicate that the extract significantly inhibits key carbohydrate-digesting enzymes. This inhibition suggests that *Delonix regia* may effectively reduce carbohydrate absorption and subsequent postprandial hyperglycaemia, thus supporting its traditional use in managing diabetes

The observed efficacy of Delonix Regia could be attributed to its rich phytochemical constituents known for their antioxidant and anti-inflammatory properties. These compounds may contribute to the extract's ability to enhance insulin signaling and mitigate oxidative stress, which is often exacerbated in diabetic conditions.

Overall, this study substantiates the traditional claims regarding *Delonix regia's* anti-diabetic effects and suggests its potential as a natural therapeutic agent. However, further research is necessary to elucidate the specific mechanisms underlying these effects and to evaluate the long-term safety and efficacy of *Delonix regia* in diabetic patients. Future studies should also explore the extract's effects on other diabetes- related complications, including nephropathy and neuropathy, to fully appreciate its therapeutic potential in holistic diabetes care.

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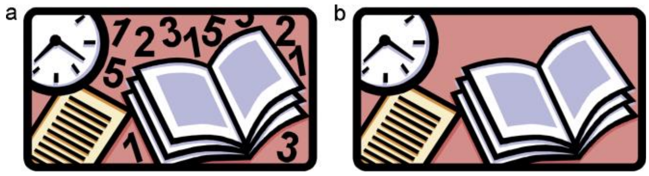

Fig. 1 - (a) first picture; (b) second picture.

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