ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

IN-VITRO ANTI HYPERGLYCEMIC EVALUATION OF HYDROALCOHOLIC EXTRACT OF *DELONIX REGIA* BARK

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ARTICLE INFO

Key words:

Antihyperglycemic activity, *Delonix regia*, hydroalcoholic extract ,*In vitro* study, GC-MS analysis.

Access this article online
Website:
https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Anti-hyperglycemic agents is a substance that helps a person with diabetes controls their level of glucose (sugar) in the blood. It includes insulin and the oral anti hyperglycemic agents. Diabetes is a metabolic disorder that is characterized by increased levels of blood glucose leading to other major complications. Thus, there prevails a necessity for obtaining these anti hyperglycemic agents through easily available flora. *Delonix regia*, is a tree cultivated across the world, has also been used as traditional medicine in various disorders. Aim of the project work was to evaluate the anti hyperglycemic activity in the hydroalcoholic extract of *Delonix regia* bark for the treatment of hyperglycemia. The collected bark was dried, powdered and extracted through cold maceration method. The extract was further concentrated to obtain a gummy mass of the hydroalcoholic extract.

The extract was subjected to phytochemical analysis through conventional chemical tests and GC-MS. After the identification of the phytoconstituents, they were studied for their clinically proven properties. *In vitro* anti hyperglycemic studies were carried out through assays like alpha-amylase inhibition assay and alpha-glucosidase inhibition assay. The results of the extract were compared with results of standard acarbose. The IC_{50} values of standard in alpha-amylase inhibition assay and α-glucosidase inhibition assay was 98.77µg/ml and 84.33µg/ml respectively. The IC_{50} values of hydroalcoholic extract of *Delonix regia* bark in alpha-amylase inhibition assay and alpha-glucosidase inhibition assay was 167µg/ml and 116.31µg/ml respectively. From the study, hydroalcoholic extract of bark of *Delonix regia* exhibit antihyperglycemic activity compared to standard acarbose.

INTRODUCTION

In the medical community, epidemics of metabolic diseases have been attributed to genetic background and changes in diet, exercise and aging factors. However, there is now evidence that other environmental factors may contribute to the rapid increase in the incidence of obesity, T_2D and other aspects observed over the past three decades.[1].

Diabetes Mellitus (DM) is a metabolic disorder characterized by the presence of chronic hyperglycemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins.[2] From a medical perspective, it represents a series of metabolic conditions associated with hyperglycemia and caused by partial or total insulin insufficiency. From a societal perspective it is a burden that the disease places on economies, in terms of costly treatment and associated premature morbidity and mortality. From the individual patient's perspective, it is a lifelong condition requiring attention to diet, lifestyle and monitoring of blood glucose with frequent administration of medication.[3]

Many treatments that involve the use of medicinal plants are recommended. Most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and have

anti-diabetic effects. The anti-hyperglycemic effects results from treatment with plants are often due to their ability to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose.[4] Literature review revealed that the various part of *Delonix regia* possess anti diabetic activity and hence this study investigates the anti hyperglycemic activity and phytochemical analysis of bark.

Delonix regia also known as the Royal Poinciana or Flamboyant is as species of flowering plants in the pea family. In some countries, *Delonix regia* has folkloric used as a medicinal agent to treat some disorders, such as constipation, inflammation, rheumatoid arthritis, diabetes, pneumonia, and malaria. The functional phytoconstituents exist in leaves, flowers, barks, and seeds of *Delonix regia* includes flavonoids, alkaloids, saponins, sterols, β-sitosterol, lupeol, tannins, carotenoids, and phenolic acids.[5]

MATERIALS AND METHODS

Collection and authentication:

The bark of *Delonix regia* were collected from the surrounding areas of Tiruppur, Tamil Nadu, India (Lattitude longitude) during the month of August and authenticated by Botanical survey of India (BSI) Southern circle, Coimbatore, Tamil Nadu. The authentication certificate number is No. BSI/SRC/5/23/2021/Tech/103. Soon after collection the bark pieces were cleaned, dried in shade and crushed to a coarse powder by mechanical mixer and sieved under mesh size 40 and 60 and stored in an airtight plastic container, until further use.

Extraction of bark material:

Coarsely powdered bark of *Delonix regia* extracted with hydroalcoholic (50% v/v Methanol) solvent in a round bottom flask for about 72 h at room temperature. After that the sediment was filtered with Whatman filter paper. The hydroalcoholic extract of *Delonix regia* (HAEDR) was further concentrated at 40°. The obtained crude extract was weighed and stored at 4° for further analysis. The percentage yield was found to be 7.8%w/w.[6]

Evaluation of phytoconstituent by GC-MS method

The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from Perkin Elmer, were engaged for analysis. The instrument was set as follows, injector port temperature set to 220^0C, interface temperature set as 250°, source kept at 220°. The oven temperature programmed as available,75°for 2 mins, 150°/min, up to 250°/min. Split ratio set as 1:12 and the injector used was split less mode.[7] The DB-5 MS capillary standard non - polar column was used whose dimensions were 0.25mm OD x 0.25µm ID x 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 550Da. The source was maintained at 220° and 4.5e -6 mtorr vacuum pressure. The ionization energy was -70eV. The data system has inbuilt libraries for searching and matching the spectrum.[8]

Identification of compounds:

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the known component was compared with the spectrum of the standard known components stored in the inbuilt library.

Evaluation of *in-vitro* anti hyperglycemic activity:

Various methods were available to investigate the hypoglycemic property of sample. In the present study the hypoglycemic property of HAEDR was evaluated by *in-vitro* methods. The hypoglycemic property could not be concluded based on the single hypoglycemic test method. It is in practice that generally several *in vitro* test procedures are carried out to conclude the hypoglycemic properties of the sample. Among various inhibition assay methods, α-amylase inhibition and α-glucosidase assays were carried out in the present study.

Procedure of α-amylase inhibition assay

Extract (various concentration) was added with 100µl of 0.02 M Na_3PO_4 buffer (pH 6.9) and 100µl of α-amylase solution (4.5 Units/ml/min) and preincubated at 25° for10 min (Worthington, 1993). Then, 100µl of 1% starch solution was added and incubated at25° for 30 mins and the reaction was stopped by the addition of 1ml of 3,5-dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The mixture was diluted with distilled water and the absorbance was measured at 540 nm. The readings were compared with control, which contains buffer instead of extract and the percentage of α-amylase enzyme inhibition was calculated.[9]

Procedure of α-glucosidase inhibition assay

Extract (various concentration) was taken with 100µl of 0.1M phosphate buffer (pH 6.9) and100µl of α-glucosidase solution (1 Unit/ml/min) and preincubated at 25° for 5 min (Worthington,1993). Then, 100µl of p-nitrophenyl-α-D- glucopyranoside (5ml) was added and incubated at 25° for 10 min. After the incubation period, the absorbance readings were recorded at 405 nm and allegorized to a control that had 100µl of buffer in place of the extract. The results were calculated on percentage basis.[10]

RESULTS

Percentage Yield of hydroalcoholic extract of *Delonix regia* bark was found to be7.8% w/w

The major phytocompounds identified in hydroalcoholic extract of *Delonix regia* bark by GC-MS analysis includes Oleic acid, Iso propyl linoleate, Trimethylsilyoxy benzene, HexaDecanoic acid, Myo-inositol 4-C-methyl, Glucopyranoside, α-D-Glucopyranoside, DL-Arabinose.[Figure 1]

Assay of α-amylase inhibition activity was found to be IC_{50} value of Acarbose=98.77µg/ml and IC_{50} value of HADER=167µg/ml.[Figure 2]

Assay of α- glucosidase inhibition activity was found to be IC_{50} value of Acarbose=84.33µg/ml and IC_{50} value of HADER=116.31µg/m.[Figure 3]

By α-amylase inhibition assay the IC_{50} values of standard acarbose and HAEDR was found to be 98.77µg/ml and 167µg/ml respectively. [Table 1]

By α- glucosidase inhibition assay the IC_{50} values of standard acarbose and HAEDR was found to be 84.33µg/ml and 84.33µg/ml respectively. [Table 2]

DISCUSSION

The current study evaluated the *in-vitro* anti hyperglycemic activity of the hydroalcoholic extract of *Delonix regia* bark. Presence of Myo-inositol 4-C-methyl and Trimethyl silyoxy benzene in HAEDR by GC-MS method indicate that the *Delonix regia* bark may have anti hyperglycemic activity. *In-vitro* studies by α-amylase inhibition assay and α-glucosidase inhibition assay of HAEDR exhibits anti hyperglycemic activity.

Hence the presence of Myo-inositol 4-C-methyl, α-amylase inhibition activity and α-glucosidase inhibition activity of HAEDR compared with acarbose shows that the *Delonixregia* bark extract may have anti hyperglycemic activity.

TABLE 1: α-AMYLASE INHIBITION ACTIVITY OF HAEDR AND STANDARD ACARBOSE

S.No	Concentration (µg/ml)	Percentage inhibition of Acarbose (Std) (%)	Percentage inhibition of HAEDR(%)
1.	0	0	0
2	20	11.7	7.1
3.	40	19.9	12.6
4.	60	31.1	18.6
5	80	39.7	24
6.	100	51.2	30.2

The above table shows the percentage α-amylase inhibition of HAEDR at various concentration compared to standard.

TABLE 2: α-GLUCOSIDASE INHIBITION ACTIVITY OF HAEDR AND STANDARDACARBOSE

S.No	Concentration (µg/ml)	Percentage inhibition of Acarbose (Std)(%)	Percentage inhibition of Extract (%)
1.	0	0	0
2.	20	16.56	10.5
3.	40	28.4	17
4.	60	37.2	25.8
5.	80	49.5	33.9
6.	100	55.4	43.7

The above table shows the percentage α-glucosidase inhibition of HAEDR at various concentration compared to standard.

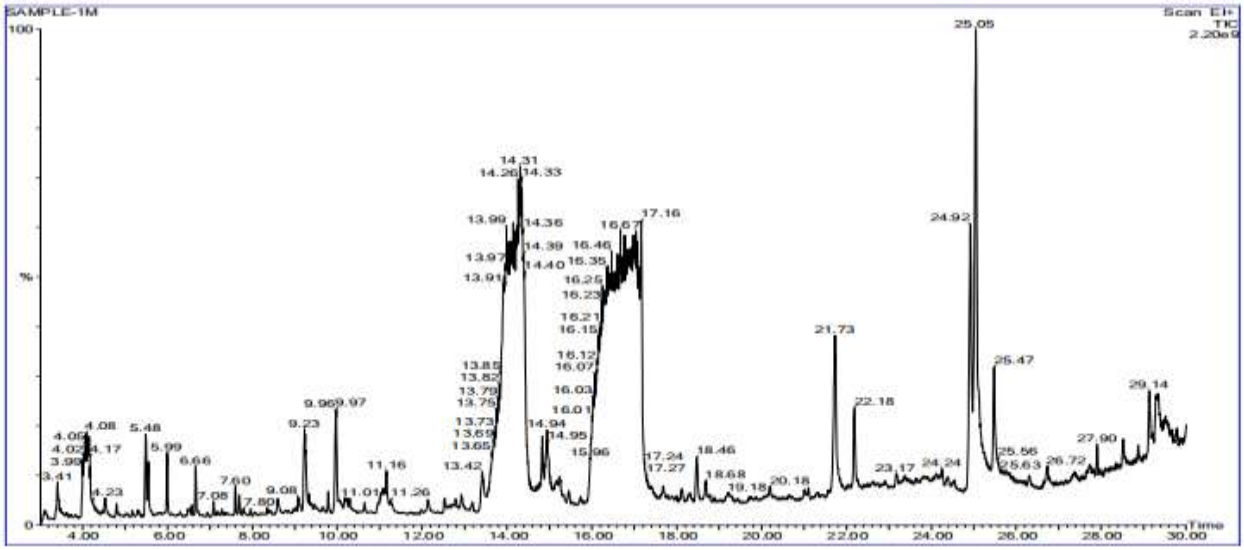

Fig 1: HAEDR GC-MS spectrum

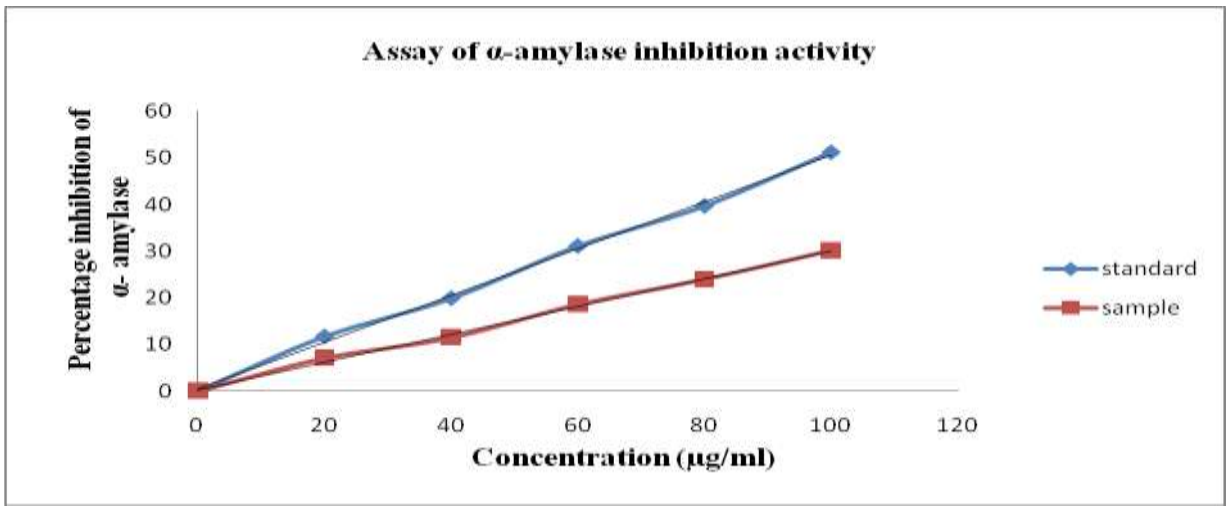

Fig 2: From the above graph IC_{50} equation was derived.

IC_{50} value of Acarbose = 84.33µg/ml (y=0.549x+3.703)

IC_{50} value of Extract = 116.31µg/ml (y=0.425x+0.566)

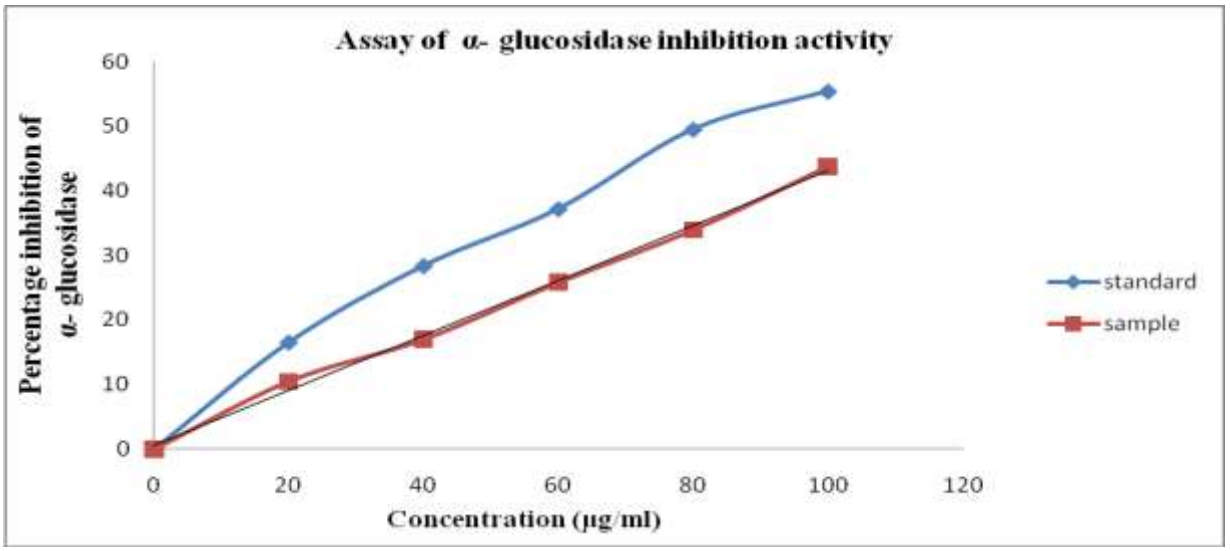

Fig 3: From the above graph IC_{50} graph was derived.

IC_{50} value of Acarbose = 84.33µg/ml (y=0.549x+3.703)
IC_{50} value of Extract = 116.31µg/ml (y=0.425x+0.566)

From the study, hydroalcoholic extract of *Delonix regia* bark may exhibit anti hyperglycemic activity, therefore *Delonix regia* bark may be useful in the treatment of hyperglycemia.

However further studies should need to identify the active principles responsible for producing anti hyperglycemic activity and the development of suitable formulation.

ACKNOWLEDGEMENT:

I thank Dr. A. Rajasekaran, Principal and Dr.G.Ariharasivakumar, Professor & HOD, KMCH College of Pharmacy who has provided excellent facilities to do research in this institution and Dr. Nalla G Palanisami, Chairman and Dr.Thavamani D Palanisami, Managing Trustee for their constant support throughout the research.

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