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RESEARCH ARTICLE

In-vitro Anti-hyperglycemic Evaluation of Hydroalcoholic Extract of *Delonix regia* Bark

Dinesh Kumar TM, Divyasankari A, Srinidhi R, Srinidhi V and Abarnadevika A*

ABSTRACT

Anti-hyperglycemic agents is a substance that helps a person with diabetes control their level of glucose (sugar) in the blood. It includes insulin and oral anti-hyperglycemic agents. Diabetes is a metabolic disorder characterized by increased blood glucose levels leading to other major complications. Thus, obtaining these anti hyperglycemic agents through easily available flora is necessary. *Delonix regia*, a tree cultivated worldwide, 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 *D. 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} standard values in alpha-amylase inhibition assay and α-glucosidase inhibition assay were 98.77 and 84.33 µg/mL, respectively. The IC_{50} values of hydroalcoholic extract of *D. regia* bark in alpha-amylase and alpha-glucosidase inhibition assay were 167 and 116.31 µg/mL, respectively. From the study, the hydroalcoholic extract of bark of *D. regia* exhibit anti-hyperglycemic activity compared to standard acarbose.

Keywords: Anti-hyperglycemic activity, *Delonix regia*, Hydroalcoholic extract, *In-vitro* study, GC-MS analysis.
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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, the disease burdens 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 blood glucose monitoring with frequent medication administration.[3]

Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India

Corresponding Author: Abarnadevika A, Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India. E-mail: srinithi88881@gmail.com

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Many treatments that involve the use of medicinal plants are recommended. Most plants have anti-diabetic effects and contain carotenoids, flavonoids, terpenoids, alkaloids, and glycosides. The anti-hyperglycemic effects that result 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 parts of *D. regia* possess anti-diabetic activity; hence, this study investigates the anti-hyperglycemic activity and phytochemical analysis of bark.

D. regia also known as the royal poinciana or flamboyant, is a species of flowering plants in the pea family. In some countries. *D. 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 *D. regia* includes flavonoids, alkaloids, saponins, sterols, β-sitosterol, lupeol, tannins, carotenoids, and phenolic acids.[5]

MATERIALS AND METHODS

Collection and Authentication

The bark of *D. regia* were collected from the surrounding areas of Tiruppur, Tamil Nadu, India (latitude longitude) during the month of August and authenticated by the 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 a mechanical mixer, sieved under mesh size 40 and 60, and stored in an air-tight plastic container until further use.

Extraction of Bark Material

Coarsely powdered bark of *D. regia* extracted with hydroalcoholic (50% v/v Methanol) solvent in a round bottom flask for about 72 hours at room temperature. After that the sediment was filtered with Whatman filter paper. The hydroalcoholic extract of *D. regia* (HAEDR) was further concentrated at 40°C. The obtained crude extract was weighed and stored at 4°C 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°C, interface temperature set as 250°C, source kept at 220°C. The oven temperature programmed as available,75° for 2 minutes, 150°/min, up to 250°/min. Split ratio set as 1:12 and the injector used was splitless mode.[7] The DB-5 MS capillary standard non-polar column was used, whose dimensions were 0.25 mm OD x 0.25 µm 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 m torr 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 InstituteStandard 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. In practice, several *in-vitro* test procedures are generally 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.

The procedure of α-amylase inhibition assay extract (various concentrations) was added with 100 µL of 0.02 M Na_3PO_4 buffer (pH 6.9) and 100 µL of α-amylase solution (4.5 units/mL/min) and preincubated at 25°C for 10 min (Worthington, 1993). Then, 100 µL of 1% starch solution was added and incubated at 25°C for 30 minutes and the addition of 1-mL of 3,5-dinitro salicylic acid reagent stopped the reaction. The test tubes were then incubated in a boiling water bath for 5 minutes 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]

The Procedure of α-glucosidase Inhibition Assay

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

RESULTS

The percentage yield of hydroalcoholic extract of *D. regia* bark was found to be7.8% w/w.

The major phytocompounds identified in the hydroalcoholic extract of *D. regia* bark by GC-MS analysis include oleic acid, isopropyl linoleate, trimethylsilyoxy benzene, hexa decanoic 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 acrabose= 98.77 µg/mL and IC_{50} value of HADER=167 µg/mL (Figure 2).

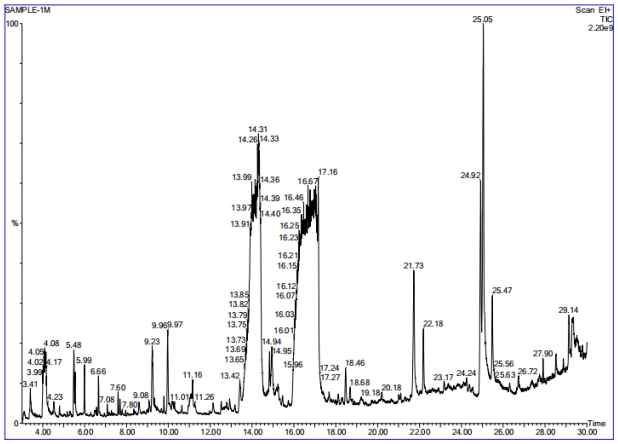

Figure 1: HAEDR GC-MS spectrum

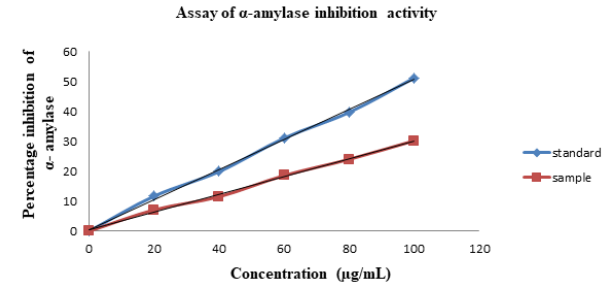

Figure 2: From the above graph IC_{50} equation was derived
IC50 value of Acarbose = 84.33 µg/mL (y=0.549x+3.703)
IC50 value of Extract = 116.31 µg/mL (y=0.425x+0.566)

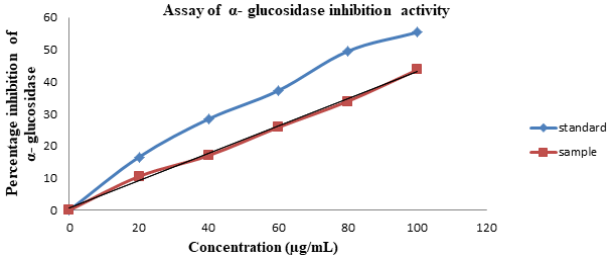

Figure 3: From the above graph IC_{50} graph was derived.
IC50 value of Acarbose = 84.33 µg/mL (y=0.549x+3.703)
IC50 value of Extract = 116.31 µg/mL (y=0.425x+0.566)

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 standard acarb

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 of α-glucosidase inhibition of HAEDR at various concentrations compared to the standard.

Assay of α- glucosidase inhibition activity was found to be IC_{50} value of acrabose=84.33 µg/mL and IC_{50} value of HADER= 116.31 µg/mL (Figure 3).

By α-amylase inhibition assay the IC_{50} values of standard acarbose and HAEDR was found to be 98.77 and 167 µg/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 84.33 µg/mL, respectively (Table 2).

DISCUSSION

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

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

However, further studies should identify the active principles responsible for producing anti-hyperglycemic activity and developing suitable formulations.

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