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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND IN-VITRO ANTIDIABETIC STUDIES OF METHANOLIC EXTRACTS OF *CASSIA TORA*

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ABSTRACT

In this study, medicinal plants, namely *Cassia tora* (Family - Caesalpiniaceae) was studied for phytochemical screening and possible effects on starch breakdown by α-amylase in vitro. The percentage yield of plant was calculated as per standard method. The yields were found to be 4.2 g (8.4% w/w of crude drug) of Methanolic extract. Total Flavonoid content was determined quercetin equivalent in MECT. Acarbose (at a concentrations 100 µg/mL) showed 58.31% inhibitory effects on the α-amylase activity with an IC_{50} value 84.19±021 µg/mL. The methanol extracts of *Cassia tora* (at a concentration 100 µg/mL) exhibited 75.31% of α-amylase inhibitory activity with an IC_{50} values 57.19±014 µg/mL. The results of study indicate that methanolic extract of *Cassia tora* plant showed appreciable α-amylase inhibitory effects.

Key words: *Cassia tora*, phytochemical screening, TFC and α-Amylase Inhibitory Assay.

INTRODUCTION:

Diabetes is a common disorder caused by a lack of insulin or insulin resistance leading to impaired glucose metabolism. Glucose is the key component used by the body's cells to make energy. In order for glucose to move from the blood into most cells insulin is required with exceptions of the brain and exercising muscles (Prasad et al., 2009; Atangwho et al., 2010). Insulin is a hormone produced by the beta cells of the pancreas and is secreted into the blood in response to the increase in blood glucose level. It is Insulin dependent diabetes mellitus where the body stops producing insulin or produces too little insulin to regulate blood glucose level. Type I diabetes comprises about 10% of total cases of diabetes in the United States. It is typically recognized in childhood or adolescence. It is used to be known as juvenile-onset diabetes or insulin dependent diabetes mellitus (IDDM). Type II diabetes mellitus is a Non–insulin

dependent (NIDDM). It is due to insulin resistance or reduced insulin sensitivity, combined with relatively reduced insulin secretion which in some cases becomes absolute. The body tries to overcome this resistance by secreting more and more insulin. At least 90% of patients with diabetes have type II diabetes (Messier *et al*., 2005; Reunanen *et al*., 2000).

Cassia tora Linn. (Family - Caesalpiniaceae) is generally distributed throughout India, Sri Lanka, West China and tropics. It is known as Charota (Hindi); Foetid Cassia (English) and Jui Ming Zi (Chinese) (Varier, 1995).

The gum powder that is obtained from seeds of cassia tora is called as "panwar gum" in Ayurveda and this gum powder is used for its emulsifying property. It is for this reason the gum powder is used for curing skin ailments and which is added in the preparation of different forms of skin medications like cream, lotion, etc.

The seeds also found to show antihelmintic activity for diseases like pheretima posthuma and ascaridia galli due to the presence of flavonoids both the extract exhibit antihelmintic activity when combined in highest concentration. The popularity of these plant seeds has necessitated requirements in pharmaceutical industry. However, when it comes to buying, it should be from a certified manufacturer and supplier and Ultrafine remains the best destination (Jain and Patil, 2010).

The aim of this work was to carried out the phytochemical screening, quantitative analysis and in-vitro anti diabetic activity by α-Amylase Inhibitory Assay.

MATERIAL AND METHODS

Collection and Authentication of Plant Drug

The leaves of selected plant namely *Cassia tora Linn* were identified and collected from Local area of Bhopal. The entire plant drug was authenticated by expert botanist of Department of Botany Geetanjali College Bhopal. The collected plant drug was cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of Plant Drug

Maceration method (Pandey and Tripathy, 2014**)**

The Collected plant drug (Leaves) was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drug was converted into moderately coarse powder in hand grinder. Powdered plant

drug was weighed (50 gm) and packed in air tight glass container. The plant Material (Leaves) was extracted with methanol for about 24 hrs with randomly shaking. Shaking of the drug during maceration is essential in order to replace the saturated layers around the drug with fresh menstrum. The liquid extract was collected in a tarred conical flask. The solvent removed by evaporating the solvent using hot plate. The dry extract obtained was weighed to calculate the percentage yield.

Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed in order to detect the various constituents present in the methanolic extracts of *Cassia tora.* The phytochemical tests were performed as per standard methods (Kokate, 1994; Harborne, 1976).

Estimation of total flavonoids content by Aluminum Chloride Colorimetric Method:

Procedure:

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 5,10,15,20 and 25 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 420 nm (λmax of quercetin) with a lab science UV-1800 spectrophotometer. Aluminum chloride, 1% and potassium acetate, 1M solutions were prepared (Shraim *et al*., 2021).

Stock Solution of Extracts: 100 mg of the plant extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions:

0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminum chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 420 nm. All prepared solutions were filtered through whatmann filter paper before measuring

In Vitro Antidiabetic Studies

In vitro α-Amylase Inhibitory Assay

The assay was carried out following the standard protocol with slight modifications (Hansawasdi *et al*., 2000). Starch azure (2 mg) was suspended in 0.2 mL of 0.5M Tris–HCl buffer (pH 6.9) containing 0.01 M $CaCl_2$ (substrate solution). The tubes containing substrate solution were boiled for 5 min and then preincubated at 37°C for 5 min.

Methanol extract of *Cassia tora* was dissolved in DMSO in order to obtain concentrations of 10, 20, 40, 60, 80, and 100 µg/ mL. Then, 0.2 mL of plant extract of particular concentration was added to the tube containing the substrate solution.

In addition, 0.1 mL of porcine pancreatic amylase in Tris–HCl buffer (2 units/ mL) was added to the tube containing the plant extract and substrate solution. The reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C.

The absorbance of resulting supernatant was measured at 595 nm using spectrophotometer (lab science 1800 UV–VIS spectrophotometer).

Acarbose, a known α-amylase inhibitor was used as a standard drug. The experiments were repeated thrice. The α-amylase inhibitory activity was calculated by using following formula:

The α-amylase inhibitory activity = (Ac+) – (Ac–) – (As – Ab)/ (Ac+) – (Ac–) × 100

where Ac+, Ac–, As, and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively. The concentration of acarbose and plant extracts required to inhibit 50% of α-amylase activity under the conditions was defined as the IC_{50} value. The α-amylase inhibitory activities of plant extracts and acarbose were calculated, and its IC_{50} values were determined.

RESULTS AND DISCUSSION:

The moderately coarse powder of *Cassia tora* (50g) leaves was extracted by maceration method subjected with methanol as a solvent. The obtained extract was dried and weighed. The percentage yield of plant was calculated as per standard method. The yields were found to be 4.2 g (8.4% w/w of crude drug) of Methanolic extract (Table 1).

Results of Phytochemical test showed the presence of various bioactive compounds such as Carbohydrates, Flavonoids and Diterpenes. Phenol alkaloids, Saponins and protein & amino acid were found to be absent in methanolic extract of *Cassia tora* (Table 2).

Table 1: Extractive values obtained from *Cassia tora* Leaves

S. No.	Solvent	Time of extraction (Hours)	Color of extract	Yield	% Yield
1	Methanol	24	Dark Brown	4.2 gm	8.4 %

Table 2: Preliminary phytochemical screening of *Cassia tora* Leaves

S. No.	Phytoconstituents	Methanolic Extract
1	Alkaloids	Absent
2	Saponins	Absent
3	Phenols	Absent
4	Carbohydrates	**Present**
5	Flavonoids	**Present**
6	Proteins & amino acids	Absent
7	Diterpenes	**Present**

Estimation of total flavonoids content

Flavonoid content was calculated from the regression equation of the standard plot ($y=0.02x+0.020$, $R^2=0.995$) and is expressed as quercetin equivalents (QE) table 4, Fig. 2. Total Flavonoid content was 0.165mg/100mg quercetin equivalent in HETP

Table 6.4 Total Flavonoid content of methanolic extract *Calendula officinalis Cassia tora*

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mg/100mg
1	Methanolic extract (100µg/ml)	0.832

n=3, values are given in SEM

Result of In-Vitro Antidiabetic activity:

Acarbose (at a concentrations 100 µg/mL) showed 58.31% inhibitory effects on the α-amylase activity with an IC_{50} value 84.19±021 µg/mL (Table 1). The methanol extracts of *Cassia tora* (at a concentration 100 µg/mL) exhibited 75.31% of α-amylase inhibitory activity with an IC_{50} values 57.19±014 µg/mL (Table 2).

Table 1: Alpha-amylase inhibitory effects of acarbose (standard α-amylase inhibitor)

Drug	Concentration (µg/mL)	% Inhibition	IC50 value (µg/mL)
Acarbose	10	18.62	84.19±021
	20	22.31	
	40	30.41	
	60	38.47	
	80	47.25	
	100	58.31	

Table 2: Alpha-amylase inhibitory effects of Methanolic extract of *Cassia tora*

Plant extract	Concentration (µg/mL)	% Inhibition	IC_{50} value (µg/mL)
Methanol	10	13.14	57.19±014
	20	36.25	
	40	49.12	
	60	64.66	

	80	70.24	
	100	75.31	

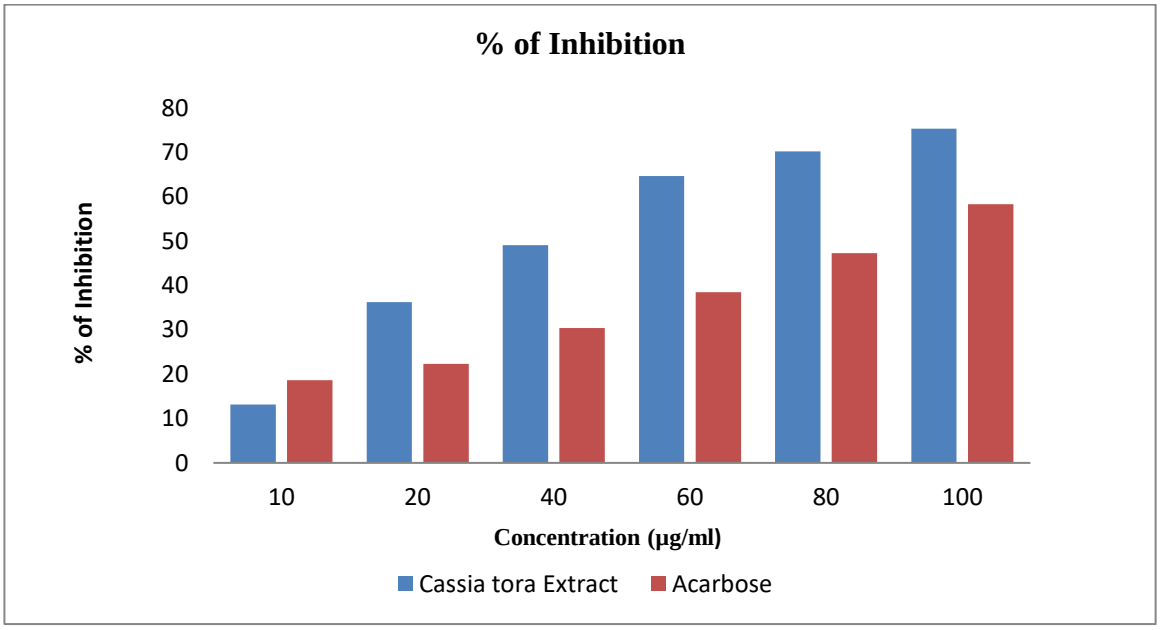

Figure 1: Percentage of α-amylase inhibitory effects of acarbose (standard drug), methanolic extracts of *Cassia tora*

Conclusion

The results of study indicate that methanolic extract of *Cassia tora* plant showed appreciable α-amylase inhibitory effects. We compared IC_{50} values of α-amylase inhibitory effects of Methanolic extract of *Cassia tora* with Acarbose (standard drug). In this study, the methanolic extract of *Cassia tora* (at a concentration 100 µg/mL) showed 75.31 % of α-amylase inhibitory activity with IC_{50} value 57.19 µg/mL. The Methanolic extract of *Cassia tora* showed appreciable α-amylase inhibitory effects when compared with acarbose. It may be due to the presence of more chemical constituents such as flavonoids (quercetin, quercetin, rutin), and alkaloids in the extract. The plant-based α-amylase inhibitor offers a prospective therapeutic approach for the management of diabetes.

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