

In vitro α -amylase Inhibition and Chromatographic isolation of *Cassia Fistula* linn. Bark

Shrikant N. Malpani^{1*} and KP. Manjunath²

¹Leben Laboratories Pvt. Ltd., L 4, Phase-III, M.I.D.C. Akola, Maharashtra, India

²Department of Pharmacognosy, K.L.E.S' College of Pharmacy, Hubli, Karnataka, India.

ABSTRACT

In the present investigation we report the inhibitory activity against digestive enzymes related to diabetes. α -amylase is the main enzyme in humans responsible for the breakdown of starch. Inhibition of these enzymes delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the post-prandial plasma glucose rise. The total alcoholic extract and its ethyl acetate and butanol fractions of the *Cassia Fistula* Linn. bark were tested for in vitro alpha amylase inhibitor activity. Ethyl acetate fraction has shown significant alpha amylase inhibition. Depending on α -amylase inhibition ethyl acetate fraction further subjected to the chromatographic isolation using gradient column chromatography which on spectral data reveals the presence of flavonoidal moiety. Therefore, our studies support the use of active constituents from the bark of *Cassia fistula* for diabetes management also established some pharmacological evidence to support the folklore claim that is used as an antidiabetic.

Keywords: digestive enzymes, α -amylase, *Cassia fistula*, ethyl acetate fraction.

INTRODUCTION

Diabetes is one of the most prevalence chronic disease in the world. This is a chronic incurable condition due to insulin deficiency that affect 10% of the population. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025. For a long time, diabetes has been treated with several medicinal plants or their extract based on the folklore medicine. Nowadays herbal medicines are highly recommended for the treatment of diabetes inspite of other therapeutic option, which can produce serious side effects & in addition they are not safe during pregnancy. Therefore the search for the more effective and safer hypoglycemic agents has continued to be an important area of active research. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agent from medicinal plant has become more important.¹⁻²

One therapeutic approach for treating diabetes is to decrease the post-prandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes like alpha amylase and alpha glucosidase,

in the digestive tract. Alpha amylase is the main enzyme in humans responsible for the breakdown of starch and more other plain sugars like dextrine, maltotriose, maltose and glucose. Although the activity of these enzymes has not been directly involved in the etiology of the diabetes, however alpha amylase inhibitor has been thought to improve glucose tolerance in diabetic patients.³⁻⁴

Cassia fistula Linn. also known as golden shower, Indian laburnum, belongs to the family Leguminosae. In traditional medicine, it is used in the treatment of hematemesis, pruritis, intestinal disorders, leucoderma, **diabetes**, & as antipyretic, analgesic & laxative.⁵⁻⁶ *Cassia fistula* is a moderate sized deciduous tree, distributed throughout India. It is 8-15m to 24m in height, with greenish grey smooth bark when young & rough, dark brown when mature. Leaflets 8-12 pair, flowers yellow, long drooping racemes. Pod cylindrical & pulpy. Seeds light brown, hard & shiny.⁷⁻⁸

As species of *Cassia* are rich sources of flavonoids, anthraquinones and polysaccharides. The flavonol and xanthone glycosides have been already reported from the bark of the plant *Cassia fistula*. So attempt has been made to isolate the

phytoconstituent responsible for alpha amylase inhibition.⁹⁻¹¹

MATERIALS AND METHODS

Plant

The bark of *Cassia fistula* L. were collected from the local areas of Hubli, Karnataka, and authenticated by Dr. B.D. Huddar, Head, Department of Botany, H.S.K. Science Institute, Hubli. A voucher specimen (No.07PG353, Shrikant Malpani) has been deposited in the PG Pharmacognosy laboratory of the college for future reference.

Preparation of the extracts

The *Cassia fistula* L. bark was shade dried at room temperature, pulverized, and extracted with 95% ethanol at temperature 40-60°C, in a Soxhlet extractor for 48 hours. The solution was evaporated giving a dark brownish residue (27.39%) stored in dessicator. The total alcoholic extract was dissolved in water & fractionated with pet ether, benzene and ethyl acetate (9.12%). All the extracts were subjected to the phytochemical screening.¹²

In-Vitro alpha amylase inhibitor activity³⁻⁴

Chemicals

- 1) Porcine pancreatic alpha amylase (EC 3.2.1.1, type VI, Sigma)
- 2) Distilled water
- 3) Potato starch
- 4) Dimethylsulphoxide (DMSO)
- 5) DNS color reagent
- 6) Maltose

Plant extract used

Total alcoholic extract and its ethyl acetate and butanol fraction were used for the study. All the extracts were prepared in DMSO to get 20 mg/ml concentration.

Enzyme solution

It was prepared in ice-cold water to get final concentration of 4 units/ml

Starch solution

It was prepared in phosphate buffer (0.5% w/v).

DNS color reagent

Solution -I) Dissolved 30gm of Sodium Potassium Tartarate Tetrahydrate in 50 ml of water.

Solution -II) Dissolved 1 gm 3,5-Dinitrosalicylic acid in 20 ml of 2 mol/lit of NaOH.

DNS reagent is freshly prepared by mixing Solution-I and II to make up the volume upto 100 ml.

Maltose standard calibration curve:¹³

Stock solution of maltose 1mg/ml prepared in phosphate buffer (pH 6.9) and dilutions were made to get final concentrations of 08, 16, 24, 32 and 40 µg/ml of the maltose. 1ml of DNS color reagent and 3 ml of the sugar solutions were taken in a test tube and placed in a boiling water bath for 5 min, cooled to room temperature and read the absorbance at 540nm against the blank.

Alpha amylase inhibition

Procedure

The following samples were prepared for the study.

Samples	Plant extract (20mg/ml)	Starch solution (0.5% w/v)	Distilled water	Enzyme solution (4 units/ml)	DMSO
Control	--	1.6 ml	640 µl	800 µl	160 µl
Blank	160 µl	1.6 ml	1.44 ml	--	--
Test	160 µl	1.6 ml	640 µl	800 µl	--

The tubes were incubated at 25 °C for a total of 3 min. Final concentrations in the incubation mixture were plant extract, 1 mg/ml, 0.25% (w/v) starch and 1 unit/ml enzyme. At intervals from addition of enzyme (0,1, 2 and 3 min), 800 µl mixture was removed and added into a separate tube containing 400 µl DNS color reagent solution and placed on a water bath at 85 °C. After 15 min, this mixture was diluted with 3.6 ml distilled water and removed from the water bath. The absorbance of the mixtures was measured at 540 nm.

Calculations

From the absorbance obtained, the % (w/v) of maltose generated was calculated from the standard

calibration curve. The % reaction and % inhibition was calculated as per the following formula:

$$\% \text{ Reaction} = \frac{\text{Mean maltose in sample}}{\text{Mean maltose in control}} \times 100$$

$$\% \text{ Inhibition} = 100 - \% \text{ Reaction}$$

All the results are shown in Table no: I and Figure No: I

CHROMATOGRAPHIC STUDIES¹⁴⁻¹⁷

The ethyl acetate fraction was found to contain three compounds chromatographically. The compounds were separated by silica gel column using different organic solvents of increasing polarity¹⁸. Compound-I was eluted with benzene: ethyl acetate (3:7 v/v), compound-II with benzene: ethyl acetate (1:9 v/v).

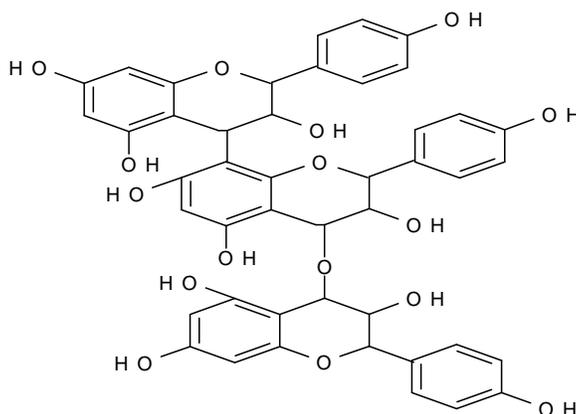
Compound-I: Rf 0.730, solvent- benzene: ethyl acetate (1:9 v/v); UV (CH₃OH): 288 nm; FT-IR 3367.65 cm⁻¹(OH Stretching) 1610.88 cm⁻¹(C=C Stretching, Aromaticity)

1160.81 cm⁻¹ (- C-O-C Stretching); ¹HNMR δ Value 2.1 – 2.8 (- CH, 9H, Alkyl-H) 6.8 – 7.8 (Ar-H, 17H, Ar-H), 8.4 – 10.4 (OH, 8H, Phenolic-H); ¹³C-NMR δ Value 21.15 – 60.15 (- C – H, Alkyl carbon), 40.13 – 79.44 (CH – OH), 144.46 – 170.61 (CH = CH, Aromatic); Mass spectra shows base peak at m/z 381 and M⁺ peak at m/z 986.

Compound-II: Rf 0.840, solvent- benzene: ethyl acetate (1:9 v/v); UV (CH₃OH):

282 nm; FT-IR 3365.72 cm⁻¹(OH Stretching), 1704.68 cm⁻¹ (C=O Stretching), 1610.29 cm⁻¹ (C=C Stretching, Aromaticity), 1369.38 cm⁻¹ (CH₃ Bending), 1163.22 cm⁻¹ (C-O-C Ether linkage); ¹HNMR δ Value 8.8 (OH, 1H, Phenolic), 6.5 – 7.5 (Ar-H, 4H), 4.8 – 5.3 (OH, 4H, Alcoholic), 3.8 – 4.8 (OCH₃, 6H), 1.0 - 2.8 (CH, 5H, Alkyl, CH₂, 2H, Alcoholic);

¹³C-NMR δ Value 29.53 – 40.47(C – H, Carbon), 78.37 – 83.13 (C – O, Carbon), 114.77 – 157.24 (C = C, Aromatic); Mass spectra shows base peak at m/z 353 and M⁺ peak at m/z 463.



Compound-I

RESULTS

Table 1: Mean absorbance, maltose formation and percentage inhibition by total alcoholic extract and its fractions

Sample	0 min	1 min	2 min	3 min	Maltose (ug/ml) at t = 3 min	Percentage Inhibition
Control	0.025	0.054	0.085	0.106	28.2	---
Tot. Alc	0.045	0.063	0.089	0.109	27.9	+1.07
EtOAc	0.041	0.057	0.083	0.102	27.2	+3.54
Butanol	0.053	0.067	0.092	0.111	29.4	-4.25

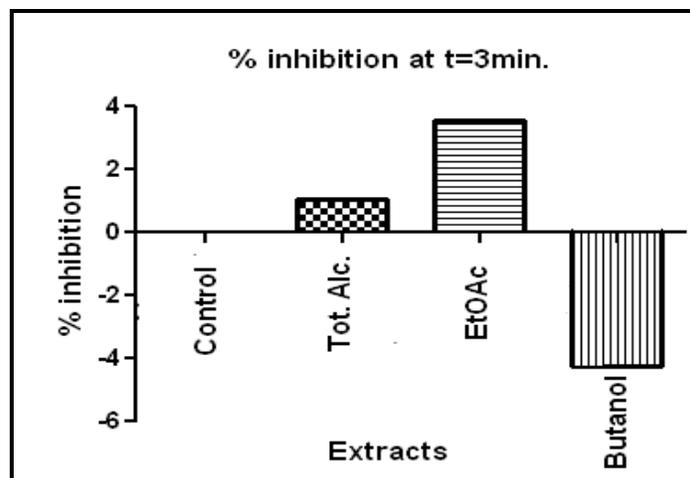


Fig. 1: Percentage inhibition of alpha amylase enzyme by various extracts at time t = 3 min

The total alcoholic extract and its ethyl acetate and butanol fractions were tested for in vitro α -amylase inhibitor activity. Ethyl acetate fraction has shown significant alpha amylase inhibition. Chromatographic isolation & spectral data of isolated Compound-I from ethyl acetate fraction was found similar to flavonoidal moiety.

The phytochemical screening of the extracts revealed the presence of tannins, flavonoids, glycosides, phenolic compounds, carbohydrates, steroids & triterpenoids.

DISCUSSION

As the prevalence of diabetes mellitus is on the increase and needs to be addressed appropriately. In this study area, herbal remedies are considered convenient for management of diabetes with postprandial hyperglycemia due to their traditional acceptability and availability, low costs, lesser side effects. In developing countries, where the per capita income is low, it is necessary to seek affordable alternative therapies. Here the ethyl acetate fraction has shown better alpha amylase inhibition as compared to the total alcoholic extract. From the collective spectral data, the isolated compound-I was found similar to flavonoidal moiety (Compound-I). Hence we can say that presence of flavonoid in the ethyl acetate fraction may be responsible for α -amylase inhibitory activity.

CONCLUSION

From the above results, it was concluded that the present study seems to support the claims by traditional medicine practitioners about the usefulness of *Cassia fistula* L. bark for the treatment of diabetes. From the phytochemical studies the correlation of antidiabetic activity can be made with the flavonoidal compounds. In

conclusion, the results from this study give scientific support to the use of *Cassia fistula* in folklore medicine for the treatment of diabetes, and show for the first time, the potential role of α -amylase inhibition in its activity.

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