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In vitro antidiabetic activity of *Tinospora cordifolia* via enzymatic assay

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Abstract
The present study evaluates the *in vitro* antidiabetic potential of *Tinospora cordifolia* Q using enzymatic inhibition assays. Inhibiting enzymes such as α-amylase and α-glucosidase is considered an effective strategy to delay glucose absorption and manage postprandial hyperglycemia. In this study, varying concentrations of *T. cordifolia* Q were tested for their inhibitory effects on these enzymes. The results revealed a concentration-dependent inhibition, indicating significant potential of the tincture in moderating carbohydrate breakdown. These findings provide scientific validation to the traditional use of *T. cordifolia* in managing diabetes and suggest that its mother tincture may serve as a promising natural adjunct for controlling hyperglycemia.

Keywords: Anti diabetic activity, enzymatic inhibition assay, *Tinospora cordifolia*

Introduction
The pervasiveness of diabetes is increasing all over the world mainly due to increased population, aging, lifestyle changes like obesity and physical inactivity. Unlike western countries, Asian countries have diabetes prevalence among middle aged peoples [1]. India has nearly 33 million diabetic individuals with the primary position in the global diabetic epidemiology map [2]. The cause of diabetes in India is multifactorial that includes genetic predisposition, lifestyle changes etc. The core aim in the management of diabetes is reaching near normal glycemia [3]. Diabetes affects mainly developing countries like India. Indeed, India presently has the largest number of diabetic patients in the world and has been infamously dubbed as the 'diabetic capital of the world' [4]. *Tinospora cordifolia* commonly called Guduchi belonging to Menispermaceae family is a large, glabrous, deciduous, climbing shrub. It is distributed throughout the tropical Indian subcontinent and China. [5] This plant is traditionally being used to treat various disease conditions like fever, dysentery, leprosy, diabetes etc [6]. The stem of the plant is generally used in treating diabetes by regulating blood glucose level. It promotes insulin secretion by inhibiting gluconeogenesis and glycogenolysis [7].

Materials and Methods
Materials used
Tinospora cordifolia Q, 3, 5 dinitrosalicylic acid colour reagent, porcine α amylase, 0.5% starch solution, microplate reader,α Glucosidase, sucrose, glucose reagent, water bath.

Fig 1: shows *Tinospora cordifolia* Q

Study design
The assay had been performed in Biogenix Research Centre, Trivandrum.

α-Amylase inhibition assay
Procedure
Three sample concentrations (250 µL/mL, 500 µL/mL, and 1000 µL/mL) of *Tinospora cordifolia* Q were prepared from the stock solution and adjusted to a final volume of 100 µL using 25 mM phosphate buffer (pH 6.9). To each, 25 µL of porcine α-amylase (HiMedia, 0.5 mg/mL) was added and the mixtures were kept at 25 °C for 10 minutes. After this, 25 µL of 0.5% starch solution (in the same buffer) was added to start the reaction, and the tubes were kept at 25 °C for another 10 minutes. The reaction was stopped by adding 50 µL of 96 mM 3,5-dinitrosalicylic acid (DNS) reagent. The mixtures were then heated in a boiling water bath for 5 minutes, cooled to room temperature, and the absorbance was read at 540 nm using a microplate reader (Erba, Lisascan) [8].

Calculation

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

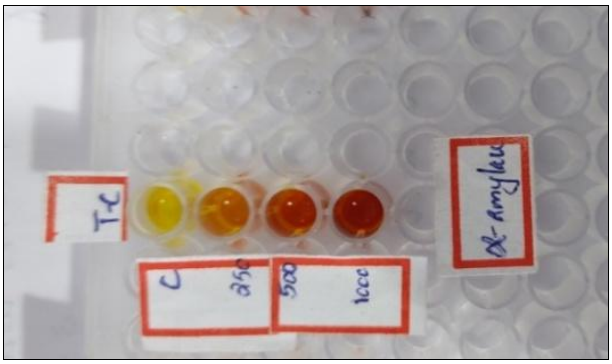

Fig 2: shows α-Amylase inhibition assay at different concentrations

α-Glucosidase inhibitionassay
Procedure
Tinospora cordifolia Q at different concentrations (250 µL/mL to 1000 µL/mL) were prepared from the stock and made up to 100 µL with 0.1 M phosphate buffer (pH 7.2). To each sample, 25 µL of α-glucosidase enzyme (Sigma-Aldrich, Lot No. 0000221279) was added, and the mixtures were incubated at 25 °C for 10 minutes. After this, 1 mL of 0.1 M phosphate buffer (pH 7.2) containing 37 mM sucrose was added. The mixtures were then incubated at 37 °C for 30 minutes. To stop the reaction, the tubes were placed in a boiling water bath for 2 minutes. A control was prepared using only phosphate buffer and enzyme (no sample). All tubes were then mixed with 250 µL of glucose reagent and incubated for 10 minutes. The absorbance was recorded at 510 nm using a microplate reader (Erba, Lisascan).[9]
Calculation

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

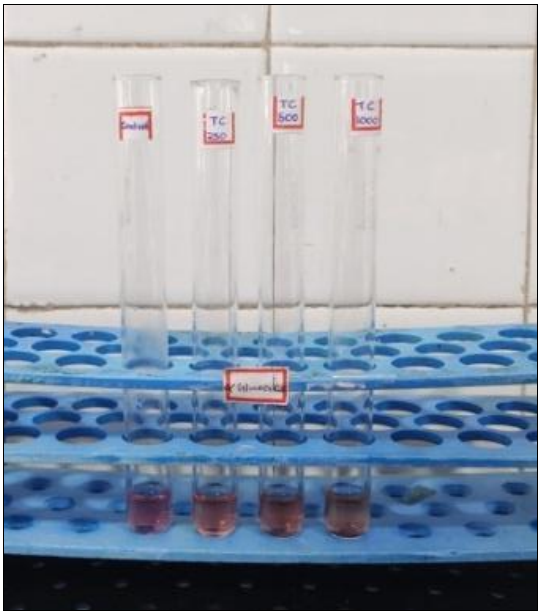

Fig 3: Shows α-Glucosidase inhibition assay at different concentrations

Observation and results
Tinospora cordifolia Q was used to assess the inhibitory activity.

Evaluation of *in-vitro* α-amylase inhibition assay of *Tinospora cordifolia*
There was increase in% inhibitory activity against α-amylase enzyme. At concentration 250µL/mL the sample showed% inhibition of 27.4567, at 500 µL/mL it was 42.7398 and at 1000 µL/mL it was 64.1537. The IC50 value of *Tinospora cordifolia* was 691.882µL/mL and was calculated by using ED50 PLUS V 1.0 Software.

Table 1: shows% inhibition of α-amylase inhibition assay

Sample concentration(µL/mL)	OD at 540nm	Percentage inhibition (%)
Control	0.3409	0.0000
Tinospora cordifolia		
250	0.2973	27.4567
500	0.1952	42.7398
1000	0.1022	64.1537

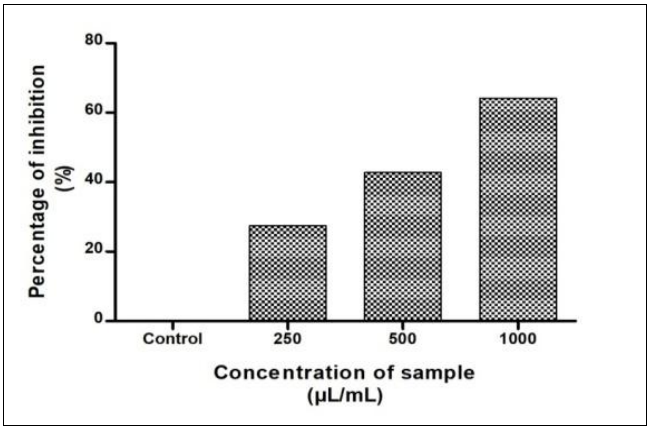

Fig 4: shows% inhibition at different concentration

Evaluation of *in-vitro* α-glucosidase inhibition assay of *Tinospora cordifolia*

Tinospora cordifolia Q showed a significant inhibitory action on α-glucosidase enzyme. The% inhibition at 250-1000 µL/mL concentration of *Tinospora cordifolia* Q showed a concentration dependent increase in% inhibition. The% inhibition varied from 63.6986 to 28.3105 for the highest to the lowest concentration. The IC50 value of TinosporaCordifolia was 668.822µL/mL and was calculated using ED50 PLUS V1.0 Software.

Table 2: shows% inhibition of α-glucosidase inhibition assay

Sample concentration (µL/mL)	Absorbance	Percentage of inhibition (%)
Control	0.438	0.0000
Tinospora cordifolia		
250	0.314	28.3105
500	0.235	46.3470
1000	0.159	63.6986

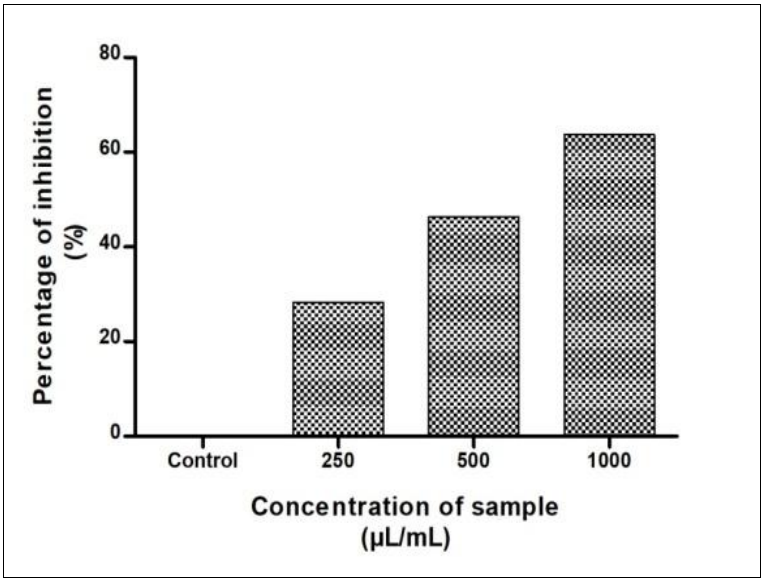

Fig 5: shows% inhibition at different concentration

Discussion

The present study aimed to evaluate the *in vitro* anti diabetic potential of *Tinospora cordifolia* Q via enzymatic inhibition assay. The findings suggest that there is notable inhibitory effect on amylase and glucosidase enzyme, indicating it's potential to modulate postprandial hyperglycemia. A dose dependent inhibition by *Tinospora cordifolia* Q with significant activity observed at higher concentrations in both amylase and glucosidase assay. This study confirms that it delays the breakdown of starch into glucose, thereby reducing glucose absorption in intestine.Most *in vitro* enzymatic studies focus on plant extracts, not regularly standardized mother tinctures. The homeopathic mother tincture likely contains similar bioactive compounds as tincture preparations in Ayurveda, but specific assay results are not widely reported. So *in vitro* enzymatic studies using mother tincture is necessary to confirm inhibitory efficacy against α-amylase, α-glucosidase, and insulin-pathway targets.

Conclusion

The study was conducted to evaluate the anti- diabetic effect of *Tinospora cordifolia* mother tincture. The *in vitro* evaluation of *Tinospora cordifolia* Q demonstrated a promising anti-diabetic potential through the inhibition of carbohydrate-hydrolyzing enzymes, specifically α-amylase and α-glucosidase. These enzymes play a critical role in the digestion of complex carbohydrates into simple sugars, which are then absorbed into the bloodstream. Inhibition of these enzymes is a well-recognized strategy for managing postprandial hyperglycemia in diabetic patients. The results of the enzymatic inhibition assays revealed that *T. cordifolia* Q showed a concentration-dependent inhibitory effect, suggesting its potential to delay glucose absorption and attenuate blood glucose levels after meals.

Conflict of Interest

Not available

Financial Support

Not available

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