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In-vitro anti diabetic activity of ethanolic extract *Quisqualis indica* Linn by using α-amylase and α-glucosidase inhibition assay

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Abstract
The present study is aimed to investigate *In-vitro* screening of anti-diabetic activity of Leaves and Flowers of *Quisqualis indica Linn* by using α-Amylase and α- Glucosidase. The powdered material was extracted by continuous hot percolation process with various solvents from non-polar to polar such as petroleum ether, aqueous and ethanol. Evaluation of in-vitro studies of anti-diabetic activity of ethanolic extracts of leaves and flowers of *Quisqualis indica Linn* by in-vitro screening of anti-diabetic activity by using Alpha Amylase and by using Alpha Glucosidase. *Quisqualis indica Linn* was found to possess significant anti-diabetic properties. The findings indicate that the above plant possess antidiabetic property in considerable level. Sothis plant can be used to develop natural drugs which may be used in lieu of commonly used strong allopathic drugs which possess a number of harmful side effects.

Keywords: *Quisqualis indica*, anti-diabetic properties, α-amylase, α- glucosidase

Introduction

Diabetes mellitus (DM) is the commonest endocrine disorder that influences in excess of 100 million individuals around the world (6% of the population) and in the following 10 years it might influence around multiple times a bigger number of individuals than it does now (WHO/Acadia, 1992; ADA, 1997). In India, the predominance pace of diabetes is evaluated to be 1-5 %. Difficulties are the significant reason for bleakness and mortality in DM. The World Health Organization (WHO) predicts that the quantity of cases worldwide for diabetes is presently 150 billion, which will twofold continuously 2025 [1]. Among the two chief types of diabetes, insulin subordinate (type~1) and non-insulin subordinate (type~2), Non-insulin subordinate diabetes represents 90% of all cases around the world, because of the body's powerlessness to react appropriately to the activity of insulin. It created by the pancreas Non-insulin subordinate diabetes is turning into a pandemic and notwithstanding the ongoing flood in new medications to treat and present the condition, its pervasiveness keeps on taking off [2-5].

Diabetes is a metabolic disorder characterized by chronic hyper glycemia that may be due to defects in insulin secretion, insulin resistance, or both [6]. Inefficient action of insulin on targeted tissues like skeletal muscle, fat tissues, and liver causes improper metabolism of carbohydrates, fat and proteins [7]. Acute and chronic complications are associated with diabetes mellitus which may lead to morbidity and mortality. The persistent elevation of blood sugar level also affects other organs such as kidneys, eyes, nerves and blood vessels [8]. One of the most leading causes of mortality worldwide is DM. Millions of people acquire this metabolic disorder every year and affects devastatingly the economy of many middle-income countries [9].

The drugs which are currently used for the treatment, may be synthetic or natural has limitations such as inadequate efficacy, high cost and also some serious side effects [10]. In spite of this the medicinal plants which are used in traditional medicines can be utilized as alternate approach for antidiabetic treatment. Nature is an abundant source of such useful medicinal plants and they are easy to collect [11]. The active constituents that can be easily isolated and some necessary chemical modifications that can be made and the techniques to make into stable formulations enhances the weightage of natural drugs in the market [12].

Here, the present study is an aimed to investigate *In-vitro* screening of anti-diabetic activity of Leaves and Flowers of *Quisqualis indica Linn* by using α-Amylase and α-Glucosidase [13-15].

Materials and Methods

Chemicals and Reagents

The dialysis membrane and 1-4, α-D-Glucan-glucanohydrolase (α-amylase) were purchased from HiMedia Laboratories, Mumbai, India. All other chemicals, reagents, kits and solvents used in this study were of analytical grade and procured locally.

Plant Profile

Botanical Name: Quisqualis indica Linn.
Local Names: English (Rangoon Creeper), Hindi (Madhumalti), Bengali (Modhumalati), Telgu (Radha Manoharam), Filipino (Niyog-niyogan), Spanish (Quiscual), China (Shih-chun-tzu), Manipuri (Parijat), Marathi (Vilayati chambeli).

Kingdom: Plantae
Division: Magnoliophyta

Class: Magnoliopsida
Order: Myrtale
Family: Combretaceae
Genus: Quisqualis
Species: Q. indica [16-19]

Habitat and Distribution: It is vining and evergreen plant which is having vigorous growth needing sturdy support and can get quite out-of-hand on its favorable growing site, it doesn't require deep and anchoring roots. It is widely distributed all over the w world especially on China, Philippines, Bangladesh, Myanmar and Malaysia and now also broadly grown in India as ornamental plant in most of the garden. Distributed over 1) Thickets and secondary forests area throughout the Philippines. 2) Ornamentally planted for its flowers. 3) Also occurs in India to Malaya. 4) Introduced in most tropical countries [20].

Plant Material and Extract Powder
The leaves and flowers of Quisqualis indica Linn were collected from Thanjavur in the month of September-2019. The plant has been taxonomically identified and authenticated by the Botanist Dr. A. Balasubramanian. The authenticated plants were used for preparation of extracts. Extract was prepared by continuous hot percolation of 300grms of powdered material in the Soxhlet apparatus. It was then extracted with various solvents from non-polar to polar such as petroleum ether, aqueous and ethanol [21].

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Pharmacological Screening Methods
Invitro Anti-Diabetic Assay (A- Amylase Inhibitory Assay) Method [22, 23, 24]
Procedure
The activity was determined using a modified assay of that described in the Worthington Enzyme Manual (Worthington, 1993; Kwon *et al*., 2006). A total of 500µL of 0.02 M sodium Phosphate buffer (pH 6.9 with 0.006 M NaCl) containing 20 mg/mL of α- amylase and varying Concentrations (25,50, 75, & 100µg) of extract as inhibitor were pre-incubated at 25ºC for 10 min.
After the pre-incubation, 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated for 10-20 min. The reaction was stopped using 1.0 mL of dinitro salicylic (DNSA) acid color reagent. The test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. Make up the volume of reaction mixture to 10mL by adding distilled water, and the absorbance was measured at 540 nm using UV-Visible light spectrophotometer. The absorbance readings of samples were compared with the control that contains buffer instead of sample extract. Varying concentrations of Acarbose (1mg/ml stock) were treated as standard.
Calculation:

$$\% \text{ ofinhibition} = \frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

α -Glucosidase Inhibitory Assay [25]
Procedure
The effect of the sample on α-glucosidase activity was determined according to the method described by Kim *et al*., using α-glucosidase from Saccharomyces cerevisiae. 200 µL of α- glucosidase (0.067 U/mL) was preincubated with different concentrations of the sample for 10 min. The substrate solution p-nitro phenyl gluco pyranoside (pNPG) was prepared in 0.1 M Sodium phosphate buffer (pH 6.9). Then 200 µL of 3.0 mM (pNPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 20 min and stopped by adding 2 mL of 0.1 M Na2CO3. The- glycosidase activity was determined by measuring the yellow-colored para nitro phenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition.

Inhibitory activity (%) = (B-T/B-C) ×100

Where, T is the absorbance of test sample, C is the absorbance of control, B is the absorbance of blank.

Results and Discussion
α-Amylase inhibitor activity of *Quisqualis indica Linn*
The alpha amylase from the three different sources that are mainly used pancreatic alpha amylase, serum alpha amylase, α-amylase from fermented body. The leaves extract showed the significant in pancreatic amylase inhibitor activity concentration range from (25 µg, 50 µg, 75 µg, 100 µg) The percentage of inhibiton was found to be (67.54%, 76.42%, 82.31%, 91.01%) respectively. The standard acarbose the concentration range (20 µg, 40 µg, 60 µg, 80 µg, 100 µg) of percentage of inhibition was found to be (13.21%, 22.29%, 39.66%, 63.10%, 81.26%) respectively.
The alpha amylase inhibition action of *Quisqualis indica Linn* may be responsible for the diabetic treatment. The α -amylase inhibition otherwise called as a starch blocker. It prevents or slows the absorption of starch in body mainly blocking the glucosidic linkage of starch and other oligosaccharides, maltose, maltriose, and other simple sugars.

α-Glucosidase Inhibitor Activity of *Quisqualis indica Linn*
α -glucosidase one of the numbers of glucosidase located in brush border surface membrane of intestinal cells and key enzymes of carbohydrates metabolism. α -glucosidase inhibition activity blocks the action of alpha glucocidase enzymes in small intestine. The rate limits they conversion

of oligosaccharides and disaccharides to mono-saccharides and it necessary for the gastrointestinal absorption.

The in-vitro alpha glucosidase inhibition activity of Ethanolic extract of leaves and flowers of *Quisqualis indica Linn*. In the various concentration (25 µg, 50 µg, 100 µg, 200 µg) and the percentage of inhibition was found to be (83.84%, 91.68%, 94.61%, 98.59%). When compared to standard acarbose concentration range (5 µg, 10 µg, 20 µg, 40 µg, 80 µg) the percentage of inhibition was found to be (28.4%, 37.9%, 56.90%, 68.24%, 79.58%) respectively.

Table 1: *In-vitro* Anti-diabetic Activity of α-amylase of Ethanolic Extract of *Quisqualis indica Linn*

Sample	Concentration (µg)	OD at 540 nm	% of inhibition
Control	-	1.402	-
Ethanolic Extract	25	0.455	67.54
	50	0.330	76.42
	75	0.248	82.31
	100	0.126	91.01

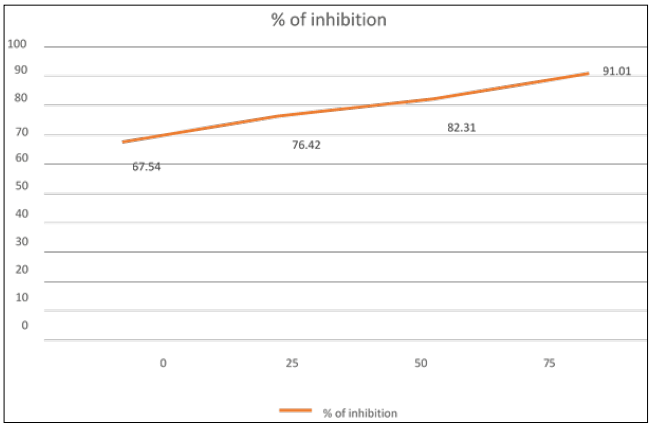

Fig 1: In-vitro Anti-diabetic Activity of α-amylase of Ethanolic Extract of *Quisqualis indica Linn*

Table 2: *In-vitro* Anti- diabetic activity of α-Amylase (Standard Acarbose) by α- Amylase Method

Standard	Concentration (µg)	OD at 540 mm	% of inhibition
control	-	0.870	-
Standard Acarbose	20	0.755	13.21
	40	0.676	22.29
	60	0.525	39.66
	80	0.321	63.10
	100	0.163	81.26

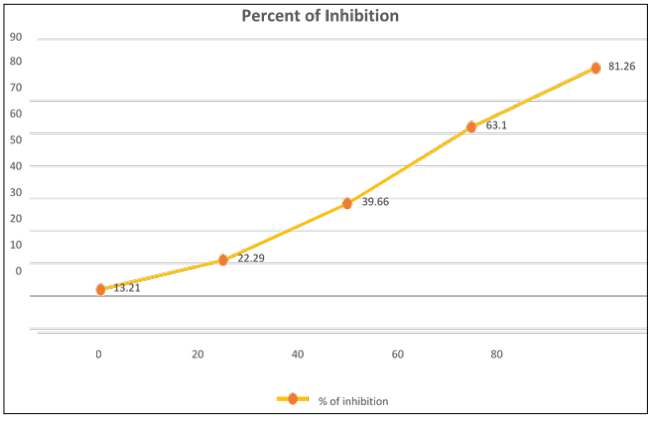

Fig 2: *In-vitro* Anti- diabetic activity of (Standard Acarbose) by using α-Amylase Method

Table 3: *In-vitro* Anti-diabetic Activity of α-Glucosidase of Ethanolic Extract of Quisqualis indica Lin

Sample	Concentration (µg)	OD at 400 mm	% of inhibition
Blank	-	0.872	-
control	-	0.019	-
Ethanolic Extract	25	0.156	83.84
	50	0.089	91.68
	100	0.064	94.61
	200	0.030	98.59

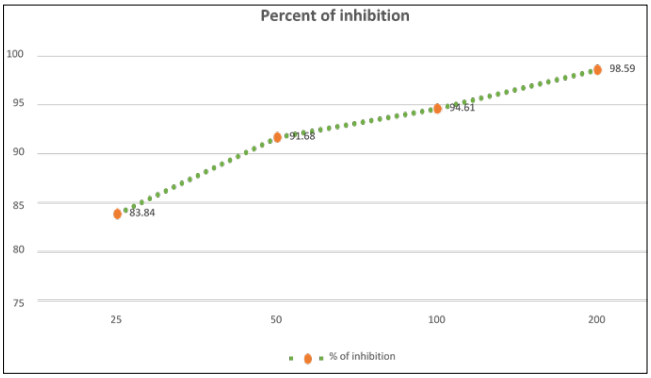

Fig 3: *In-vitro* Anti-Diabetic Activity of α-Glucosidase Inhibitor Assay (Sample)

Table 4: *In-vitro* Anti-diabetic Activity of α-Glucosidase Inhibitor Assay (Standard)

Standdard	Concentration (µg)	OD at 400mm	% of inhibition
Blank	-	0.541	-
control	-	0.056	-
Standard Acarbose	5	0.403	28.4
	10	0.357	37.9
	20	0.265	56.90
	40	0.210	68.24
	80	0.155	79.58

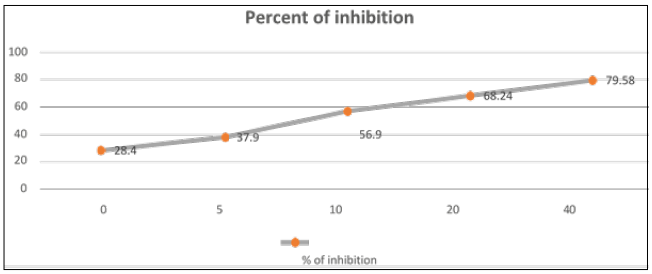

Fig 4: *In-vitro* Anti-diabetic Activity of α-Glucosidase Inhibitor Assay (Standard)

Conclusion

From the results, we concluded the ethanolic extract of leaves and flowers of Quisqualis *indica Linn* exhibited good anti-diabetic activity by ability to inhibit the α-alpha amylase and α-Glucosidase. Further our studies support they could be useful from the management in diabetes.

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