

In Vitro Evaluation of Antidiabetic Activity of Stem Extracts of *Oroxylum Indicum*

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Abstract

Background: It is estimated 180 million people in the world have diabetes mellitus. The ethnobotanical information reports about 1000 plants that may possess antidiabetic activity. Roots, leaves, seeds, fruits and stems of *Oroxylum indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine.,

Aim: The current study designed In vitro evaluation of antidiabetic activity of aqueous and methanolic stem extracts of *Oroxylum indicum*.

Materials & Methods: About 100, 200, 300, 400, 500 µg concentrations of acarbose, aqueous, and ethanolic stem extracts of *O. indicum* were used for the study. The absorbance values were taken in spectrophotometer at 540 nm and 546 nm for α -amylase and α -glucosidase enzyme, respectively.

Results: Dose-dependent % inhibition of α -amylase and α -glucosidase enzymes are observed with the both extracts. However, compare with aqueous extract, methanolic extract shows more % inhibition.

Conclusion: In this study, aqueous and methanolic stem extracts of *O. indicum* showed the in vitro antidiabetic activity.

Keywords: *Oroxylum indicum*, In Vitro Antidiabetic activity, α -glucosidase, α -amylase, Acarbose

Introduction

Diabetes is metabolic disorder characterized by hyperglycaemia and the body is unable to maintain adequate insulin secretion. It is estimated 180 million people in the world have DM. That's roughly 6% of the world population. These numbers are estimated to double by 2030.^[1] The World Health Organization estimates that 80% of the world's population relies on herbal medicine.^[2] The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice from time immemorial.^[3] Alternative systems of medicine based on plant extracts have thrived through the ages and are still practiced by a large population for the management of diabetes.^[4] The world health organization expert committee on diabetes also suggested that medicinal herbs be further investigated as they are repetitively considered to be less toxic and side effects.^[5] *Oroxylum indicum* (Bignoniaceae) also known as 'Sonapatha' is an important herb in Ayurvedic medicine and indigenous medical system for over thousands of years.^[6] Roots, leaves and stems of *Oroxylum indicum* have been used as a single drug or as a component of certain compound drug preparations in the Indian Ayurvedic system of medicine for treatment of various disorders as well as used as a tonic and Rasayana drug.^[7,8] Leaves are used externally to treat an enlarged spleen and also to alleviate headaches and ulcers and also reported for its analgesic and antimicrobial activity.^[9,10] The leaves have been reported containing flavones and their glycosides baicalein and scutellarein. Leaves also contain an anthraquinone, aloe-emodin.^[11,12,13]

Materials & Methods

Plant Materials

The fresh leaves *Oroxylum indicum* were collected in the month of December 2012, from Rajkot, Gujarat, India. The plant was then taxonomic identified in Botany Department, Shri M & N Virani Science College, Rajkot and Gujarat, India. A voucher specimen number AIP/20/02 has been deposited in herbarium department.

Preparation of stem extracts

Leaves of *Oroxylum indicum* were dried in shadow and pulverized. One hundred grams of powdered leaves were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether, toluene, chloroform and methanol. Before each extraction the powdered material was dried in hot air-oven below 50° C. Finally, marc was digested with distilled water for 24 hours to obtain the aqueous extract. Each extract was concentrated by distilling off the solvent and then vaporizing to dryness on the water-bath. Extracts were weighed and percentage was calculated in terms of the air-dried weight of the drug powder material. The various extracts of *Oroxylum indicum* were subjected to phytochemical screening. ^[14]

Inhibition of α -amylase Enzyme ^[15,16]

α -amylase (0.5 mg/ml) was mixed with the sample at various concentrations (100-500 μ g/ml) to which 1% of starch solution and 100 μ l of 0.2 M phosphate buffer (pH -6.9) were added. The reaction was allowed to be carried out at 37°C for 5 min and terminated by addition of 2 ml of 3, 5-dinitrosalicylic acid reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. α -amylase activity was determined by measuring color intensity at 540 nm in spectrophotometer.

Inhibition of α -glucosidases Enzyme ^[17]

The inhibitory activity was determined by incubating 1 ml of starch solution (2% w/v maltose) with 0.2 M tris buffer (pH 8) and various concentration of sample (100-500 mg/ml). The reaction mixture was incubated at 37°C for 10 min. The reaction was initiated by adding 1 ml of α -glucosidase enzyme (1 U/ml) to it and incubation at 35°C for 40 min. Then the reaction was terminated by the addition of 2 ml of 6 N HCl. The intensity of the color was measured at 540 nm in spectrophotometer.

The results were expressed as % inhibition using the formula:

$$\% \text{ Inhibitory activity} = (\text{Ac}-\text{As})/\text{Ac} \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

The inhibitory concentration (IC₅₀) value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. The IC₅₀ values were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

Statistical Analysis

All determinations were done in 5 times and values are expressed as the mean \pm standard error of the mean. The IC₅₀ values were calculated from plots of log concentration of inhibitor concentration versus percentage inhibition curves.

Results

α -amylase Inhibitory Activity

Aqueous stem extract of 100, 200, 300, 400, 500, 600 μ g doses inhibits the α -amylase enzyme by 9.16%, 15.03%, 23.47%, 36.89%, 47.50% and 56.59%, respectively.

Methanolic stem extract of 100, 200, 300, 400, 500 μ g doses inhibits the α -amylase enzyme by 28.69%, 44.17%, 56.24%, 64.76% and 75.57% respectively.

Acarbose of 100, 200, 300, 400, 500 μ g doses inhibits the α -amylase enzyme by 58.71%, 62.31%, 65.59%, 72.05% and 84.57%, respectively. (Figure 1)

Dose-dependent % inhibition of α -amylase enzyme is observed with the both extracts. The IC₅₀ values of aqueous extract, methanolic extract & acarbose were 530.12 \pm 15.34, 266.71 \pm 4.56, 85.02 \pm 1.12. However, compare with aqueous extract, methanolic extract shows more % inhibition of α -amylase enzyme.

α -glucosidase Inhibitory Activity

Aqueous stem extract of 100, 200, 300, 400, 500, 600 μ g doses 9.48%, 16.38%, 23.28%, 37.07% and 47.41% inhibits the α -glucosidase enzyme by respectively.

Methanolic stem extract of 100, 200, 300, 400, 500 µg doses 30.15%, 49.23%, 56.32%, 68.23% and 78.93% inhibits the α-glucosidase enzyme by, respectively.

Acarbose of 100, 200, 300, 400, 500 µg doses inhibits the α-glucosidase enzyme by 58.71%, 62.31%, 65.59%, 72.05% and 84.57%, respectively. (Figure 2)

Dose-dependent % inhibition of α-glucosidase enzyme is observed with the both extracts. The IC₅₀ values of aqueous extract, methanolic extract & acarbose were 545.77±77, 246.34±34, 89.75±1.07. However, compare with aqueous extract, methanolic extract shows more % inhibition of α-glucosidase enzyme.

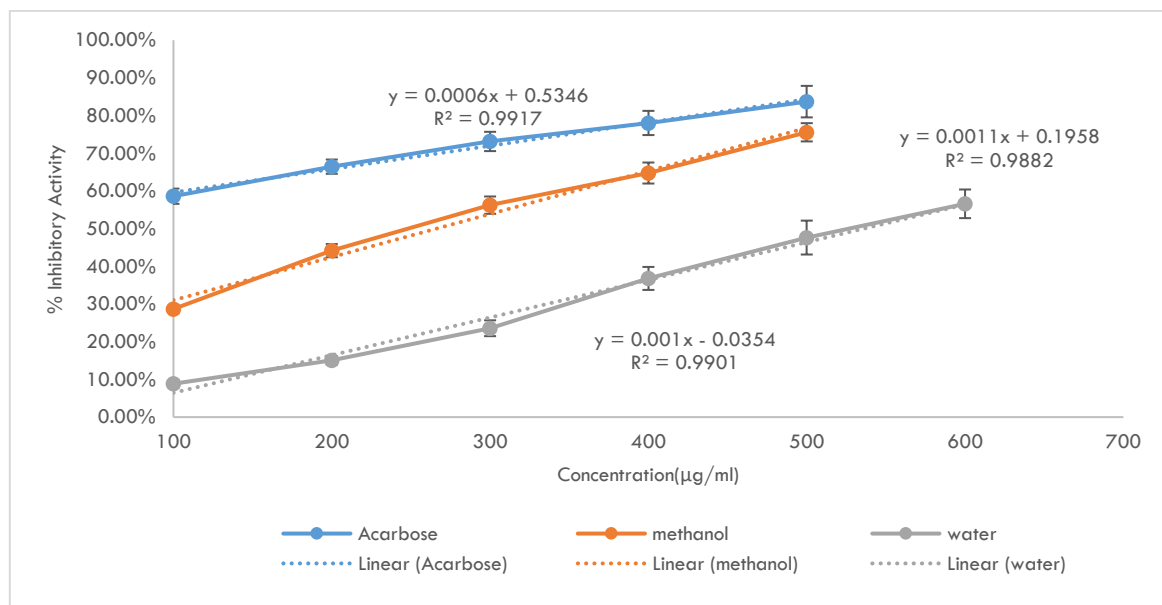


Figure 1: In vitro α-amylase inhibitory activity of stem extract of *O. indicum*

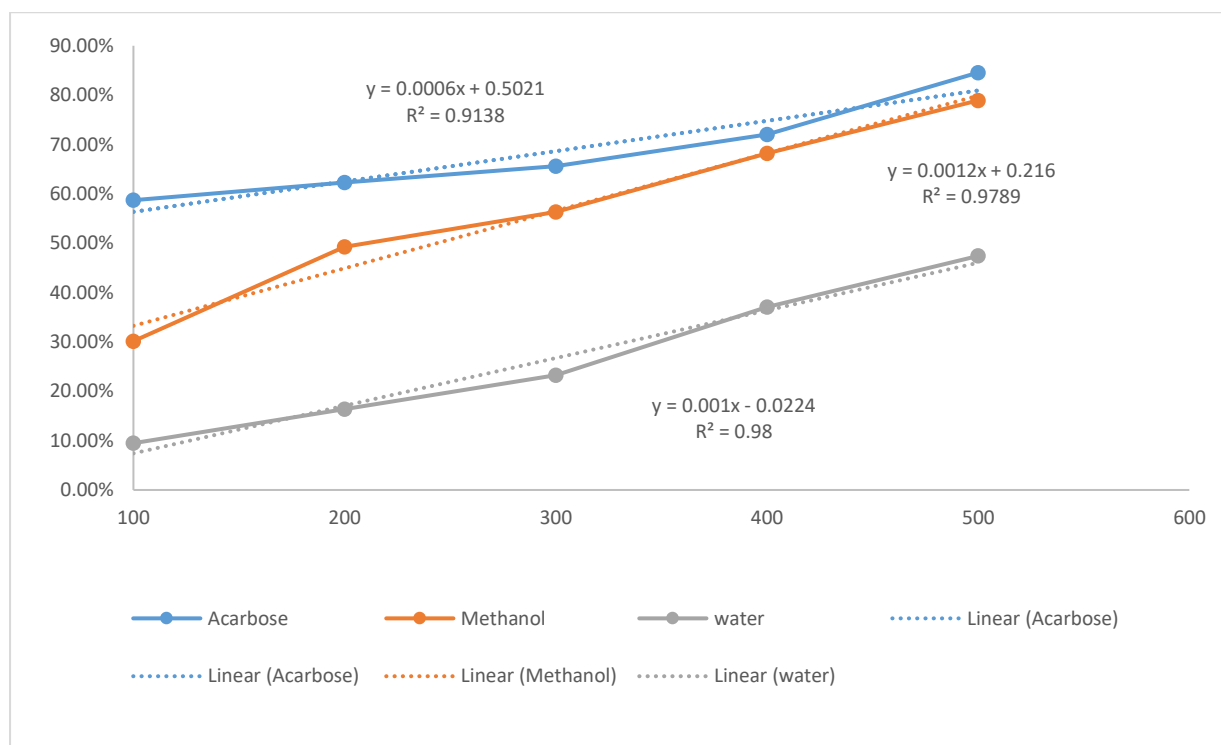


Figure 2: In vitro α-glucosidase inhibitory activity of stem extract of *O. indicum*

Phytochemical investigation of stem extracts

| Plant constituent | Methanol Extract | Aqueous Extract |
|-------------------|------------------|-----------------|
| 1) Alkaloids | -ve | -ve |
| 2) Triterpenoids | -ve | -ve |
| 3) Saponins | -ve | -ve |
| 4) Flavonoids | +Ve | +Ve |
| 5) Phenolic | +Ve | +Ve |
| 6) carbohydrates | +Ve | +Ve |

Table 1: Phytochemical study of methanolic and aqueous stem extracts of *O. indicum***Discussion**

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and comorbidities such as obesity and hypertension.^[18] It is well known that inhibition of intestinal α -glucosidase and pancreatic α -amylase activity results in delaying carbohydrate digestion of absorbable monosaccharides, causing reduction of postprandial hyperglycemia.^[19] The beneficial methods for controlling postprandial hyperglycemia in diabetic patient is to prevent or decreasing absorption of sugar after meal. Complex starches, oligosaccharides, and disaccharides must be broken down into monosaccharides by α -amylase and α -glucosidases before they are absorbed in the duodenum and upper jejunum.^[20,21] α -glucosidase inhibitors reduce intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α -glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates from the GI tract and decreases the rate of rise of postprandial glucose (PP hyperglycemia). This delay digestion and breakdown of starch may have beneficial effects on insulin resistance and glycemic index control in people with diabetes.^[22] Acarbose is α -glucosidase inhibitor which reduces digestion of complex carbohydrates and slows their absorption from the gut. These drugs also increase the release of the glucoregulatory hormone glucagon-like peptide-1 into the circulation, which may contribute to their glucose-lowering effects.^[23] However, they may cause side effect such as malabsorption, abdominal pain, flatulence, and diarrhea which lead to a high discontinuation rate.^[24] Acarbose and miglitol should not be prescribed in individuals with renal impairment. Acarbose should be used with caution in patients with hepatic diseases because it may cause reversible elevation of hepatic enzymes.^[25,26]

Experimental results showed that both extracts significantly inhibited the α -glucosidase and α -amylase enzymes. However, Methanolic extract showed more inhibitory activity than aqueous extract. The search for a new group of agents from natural resources especially from plant medicines become an attractive approach for the treatment of postprandial hyperglycemia. Flavonoids, tannins, and phenolic acids are a major group of polyphenolic compounds that have been reported to possess inhibitory activity against α -amylase.^[27,28]

The phytochemical investigation of the stem extracts was found to rich in polyphenolic components such as flavonoids which suggests that the bioactive exerting the inhibitory effect against α -amylase may be present in all plant extracts at different concentration.

Conclusion

In this study, aqueous and methanolic stem extracts of *Oroxylum indicum* showed the *in vitro* antidiabetic activity.

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Conflict of interest

I have no conflicts of interest regarding this investigation of research work.

Author's Contribution

1. Mr. Falgun Dhabaliya (studied Ph. D Scholar)
2. Dr. Mital Manvar (Ph. D. Supervisor)

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