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Research Article

# IN VITRO α-AMYLASE AND α-GLUCOSIDASE INHIBITOR ACTIVITY OF *ABUTILON INDICUM* LEAVES

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**ABSTRACT**

Postprandial hyperglycemia caused by the hydrolysis of carbohydrates by pancreatic α-amylase and intestinal α-glucosidase is a serious condition and inhibition of these enzymes is an effective way in the management of diabetes mellitus. *Abutilon indicum* (L.) Sweet leaves were used to determine their in vitro α-amylase and α-glucosidase inhibitory activity. Various concentrations from 10 µg $ml^{-1}$ to 160 µg $ml^{-1}$ were prepared in methanol along with the standard antidiabetic drug, acarbose. There was a dose dependent percent inhibition by the extract against α-amylase (7.12% - 41.31%) and α-glucosidase (8.01% - 36.13%). Logarithmic regression analysis revealed the $IC_{50}$ of 129.89 µg $ml^{-1}$ (α-amylase) and 137.61 µg $ml^{-1}$ (α-glucosidase) with a potency and preference for α-amylase over α-glucosidase inhibition by the *A. indicum* leaf extract.

**Keywords:** *Abutilon indicum*, antidiabetic, α-amylase, α-glucosidase

## INTRODUCTION

Diabetes mellitus is characterized by increased blood glucose levels resulting from absolute or lack of insulin secretion. Hydrolysis of starch by pancreatic α-amylase and uptake of glucose by intestinal α-glucosidase causes sudden rise in blood glucose levels or hyperglycemia in type 2 diabetes patients. Inhibition of these enzymes is an effective strategy for type 2 disease management [1]. Synthetic hypoglycemic agents are reported to cause gastrointestinal side effects [2, 3] and herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects [4, 5]. Medicinal plants are used in the treatment of diabetes mellitus and were the only options before the discovery of insulin based on traditional practices [6].

*Abutilon indicum* (L.) Sweet belongs to the family Malvaceae is commonly called as country mallow. The leaves are evergreen, stipulate, fili form, ovate, acuminate, toothed and rarely subtrilobate. Petiole is cylindrical form, yellowish in colour, stellate and hairy. The flowers are yellow in color, peduncle jointed above the middle; pedicels jointed very near to top and the seeds are kidney shaped, tubercled or minutely stellate hairy, black or dark brown in colour [7, 8].

Various part of the plant has been reported to have medicinal properties that include analgesic [9] (Sharma *et al.,* 1989), anti-inflammatory [10, 11], antimicrobial [12, 13, 14], antidiarrhoeal [15], antimalarial [16], hepatoprotective [17, 18], antioxidant [19, 20] and antifertility [21] activities. Leaves of *A. indicum* have reported to possess hypoglycemic [22] and antidiabetic activities [23, 24].

Natural inhibitors of carbohydrate degrading enzymes especially from plant sources offer an attractive strategy for the control of postprandial hyperglycemia. This effort has been directed at investigating α-amylase and α-glucosidase inhibitors from *Abutilon indicum* leaves to reduce the progression of diabetes.

### Materials and Methods

### Preparation of plant extract

Leaves from *Abutilon indicum* were air-dried, pulverized and extracted with methanol (1:4 w/v) at 30°C for 24 hrs. The extract was filtered, centrifuged and the residue was subjected to subsequent extraction with the same solvent and stored at -20°C.

### Phytochemical testing

The extract was subjected to preliminary qualitative tests to identify the phytoconstituents present in the leaves [25, 26].

### α-amylase inhibition assay

The α-amylase inhibitory activity was determined by following the method of Bernfield [27]. 500 µl each of *A. indcum* methanolic leaf extract and 0.02 mol $l^{-1}$ sodium phosphate buffer (pH 6.9 with 0.006 mol $l^{-1}$ NaCl) containing porcine pancreatic α-amylase (0.5 mg $ml^{-1}$) were incubated at 25°C for 10 minutes. This was followed by addition of 500 µl of starch solution (1%) in 0.02 mol $l^{-1}$ sodium phosphate buffer to the reacting mixture and was incubated at 25°C for 10 min. The reaction was stopped with the addition of 1.0 ml of dinitrosalicylic acid followed by incubating in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water and absorbance measured at 540 nm in UV-Vis spectrophotometer.

### α-glucosidase inhibition assay

The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis *et al.,* [28]. 50 µl of the extract and 100 µl of yeast α-glucosidase solution were incubated at 25°C for 10 min followed by the addition of 50 µl of 5 mM $l^{-1}$ ρ-nitrophenyl- α-D-glucopyranoside solution in 0.1 mol $l^{-1}$ phosphate buffer (pH 6.9). The reacting mixture was then incubated at 25°C for 5 min and the absorbance was read at 405 nm. Acarbose was used a positive control and the inhibitory activity of α-amylase and α-glucosidase were calculated by using the following formula,

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$$

The $IC_{50}$ values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of percent inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

## RESULTS

Phytochemical screening of *A. indicum* leaf extracts revealed the presence of alkaloids, flavonoids, phenolics, saponins, steroids, glycosides and carbohydrates (Table 1). Aminoacids, anthraquinones, proteins and tannins were absent in the extract.

**Table1: Phytochemical analysis of *A. indicum* leaf extract**

| Phytoconstituents | Test | Methanolic extract |
|---|---|---|
| Alkaloids | Mayer's test | + |
| Aminoacids | Millon's test | - |
| Anthraquinones | Borntrager's test | - |
| Carbohydrates | Molish's test | + |
| Flavonoids | Ammonia test | + |
| Glycosides | Keller Kiliani test | + |
| Phenolics | Ferric chloride test | + |
| Proteins | Millon's test | - |
| Saponins | Froth test | + |
| Steroids | Liebermann-Burchard's test | + |
| Tannins | Ferric chloride test | - |

(+) positive; (-) negative

Increasing concentrations (10, 20, 40, 80 and 160 µg ml$^{-1}$) of *A. indicum* methanolic leaf extracts were prepared for their inhibitory enzyme activities. Figure 1 and 2 shows the % inhibition of α-amylase and α-glucosidase activity of *A. indicum* methanolic leaf extract along with the standard, acarbose. There was a dose-dependent increase in percentage inhibitory activity against α-amylase with 7.12% at a concentration of 10 µg ml$^{-1}$ and 41.31% at 160 µg/ml concentration. In the case of α-glucosidase, 8.01% and 36.13% inhibition was observed at 10 µg ml$^{-1}$ and 160 µg ml$^{-1}$ concentrations. The positive control, acarbose has exerted the highest potent inhibitory action against α-amylase (66.62%) and α-glucosidase (60.62%).

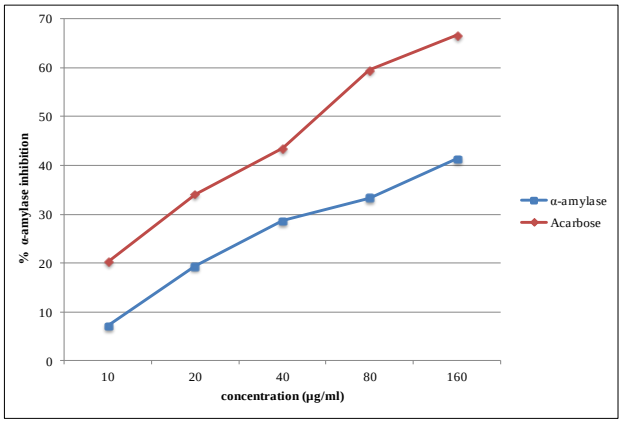


**Fig 1: α-amylase inhibitory activity of *A. indicum* leaf extract**

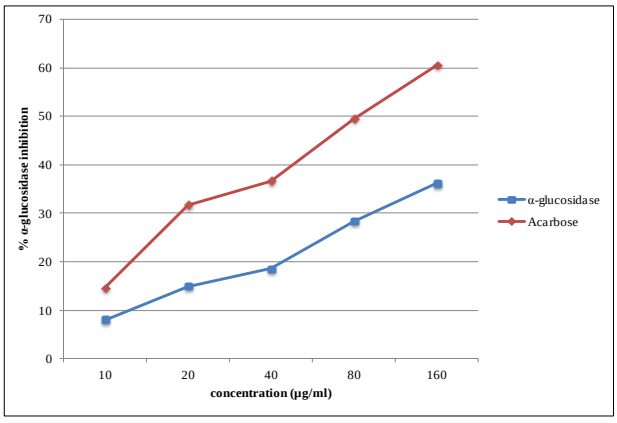


**Fig 2: α-glucosidase inhibitory activity of *A. indicum* leaf extract**

Our in vitro studies demonstrated an appreciable α-amylase inhibition with an $IC_{50}$ value of 129.89 µg ml $^{-1}$ and a weak α-glucosidase inhibitory with an $IC_{50}$ value of 137.61 µg ml$^{-1}$ activity of *A. indicum* leaf extract.

## DISCUSSION

Many bioactive principles from plants have been reported to have hypoglycemic effect [4, 29] which includes alkaloids [30, 31], flavonoids [32, 33], phenolics [34], triterpenoids [35] and carbohydrates [36]. In this study, alkaloids, flavonoids, phenolics, steroids and carbohydrates were identified from the *A. indicum* methanolic leaf extracts. A number of other phytochemicals have been identified from the leaves of *A. indicum* in earlier studies [37, 38, 24]. There is a positive correlation between plant phenolics and antidiabetic activities [39] and the presence of phenolics in *A. indicum* leaves might have influenced its hypoglycemic activity.

α-amylase catalyzes the hydrolysis of starch and α-glucosidase catalyzes the final step in carbohydrate digestion which leads to postprandial hyperglycemia. Inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion and causing reduced glucose absorption rate which consequently reduce the postprandial plasma glucose rise [40]. These inhibitors have been found useful in the control of diabetes mellitus over many years [41, 42]. Many scientists have investigated the plants containing various phytochemicals that exhibit additive and synergistic interaction in antidiabetic properties which exert positive health-promoting effects [43]. In this present study, in vitro α-amylase and α-glucosidase inhibitor activity of methanolic extract of *Abutilon indicum* (L.) Sweet leaves were evaluated. The retardation and delay of carbohydrate absorption with a plant-based α-amylase and α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus. The $IC_{50}$ values show that *A. indicum* has a potency and preference for α-amylase over α-glucosidase inhibition. A significant inhibitory activity was observed against α-amylase (7.12 - 41.31 µg ml$^{-1}$) at different concentrations than the α-glucosidase inhibition (8.01 - 36.13 µg ml$^{-1}$). The different inhibition kinetics observed between α-amylase and α-glucosidase could be due to structural differences related to the origins of the enzymes [44].

## CONCLUSION

This study justifies the hypoglycemic activity of *Abutilon indicum* leaves and can be used in the management of diabetes. However, the active principles responsible for inhibitory action of α-amylase and α-glucosidase need to be identified and characterized for the development of indigenous botanical resources for novel hypoglycemic drug development.

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