



ISOLATION OF AN ALPHA AMYLASE INHIBITOR FROM *ABUTILON INDICUM* LEAVES

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ABSTRACT

Recently we have shown that ethanol extract of *Abutilon indicum* Linn. (*A. indicum* L.) leaves of rainy season has maximum *in vitro* alpha amylase inhibitory activity. Aim of the present work was to isolate alpha amylase inhibitor from *A. indicum* L. leaves. *A. indicum* L. leaves were collected from the medicinal plant garden of North Bengal University, Siliguri, West Bengal and identified by the taxonomist. Ethanol extract of the plant leaves was processed for isolation work by standard techniques. Solvent extraction and acid hydrolysis were done followed by solvent treatment, chromatographic experiments and crystallization. A compound was crystallized. *In vitro* alpha amylase inhibitory activity of the isolated compound was checked by standard method. Acarbose, an alpha amylase inhibitor, was used as control. Results showed that the isolated compound had strong alpha amylase inhibitory activity which was comparable to that of acarbose. The isolated compound, therefore, may be used in the management of diabetes.

KEYWORDS: *Abutilon indicum* Linn. Leaves, isolated compound, alpha amylase inhibitory activity, acarbose, diabetes

1. INTRODUCTION

Rowley *et al.* wrote in their paper^[1], *The prevalence of diabetes (type 2 diabetes and type 1 diabetes) will increase by 54% to more than 54.9 million Americans between 2015 and 2030; annual deaths attributed to diabetes will climb by 38% to 385,800; and total annual medical and societal costs related to diabetes will increase 53% to more than \$622 billion by 2030*

Diabetes mellitus is mainly of two types - Type - 1 and Type-2. Type - 2 diabetes mellitus is characterized by postprandial hyperglycemia. One of the therapeutic approaches of Type - 2 diabetes mellitus, therefore, is to reduce postprandial hyperglycemia.^[2] This can be done by inhibiting carbohydrate splitting enzymes. One such enzyme is alpha amylase which hydrolyses complex carbohydrates of food to free sugars. Inhibition of alpha amylase activity reduces hydrolysis of complex carbohydrate thereby postprandial hyperglycemia may be kept under control.^[3] Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of Type - 2 diabetes mellitus.^[4] Still search is going on for more alpha amylase inhibitors. In this context medicinal plants were investigated and many plants are now-a-days known having alpha amylase inhibitory activity.^[5]

Recently we observed that ethanol extract of *A. indicum* L. leaves of rainy season has maximum *in vitro* alpha amylase inhibitory activity. Results are under communication. Aim of the present study was to isolate alpha amylase inhibitor from *A. indicum* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

2.1 Plant material

A. indicum L. leaves were collected from the medicinal plant garden of the North Bengal University, Dist. Darjeeling, West Bengal, India during rainy season (June - August). Rainy season was chosen as we observed that *A. indicum* L. leaves of rainy season has maximum *in vitro* alpha amylase inhibitory activity. Leaves were authenticated by the experts of the department of Botany of the said University. A voucher specimen (No. SM-MB-012/19) was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of the Sikkim Manipal University, Gangtok, Sikkim, India for future references.

2.2 Preparation of plant materials

Leaves of *A. indicum* L. were washed thoroughly, shed dried and powdered. The powder, used as test drug, was stored desiccated at 4 °C until further use.



Abutilon indicum Linn.

2.3 Chemicals

Chemicals required for the study were purchased from Loba Chem. Lab, Himedia Lab, India and from Merck, Germany and Sigma Chemicals Co., USA.

2.4 Isolation of alpha amylase inhibitor from *A. indicum* L. Leaves

Applying principles of standard isolation procedures of chemical compounds from plant sources^[6,7], isolation was done by the following scheme.

Diagrammatic scheme for isolation of alpha amylase inhibitor from *A. indicum* L. leaves.

Powdered leaves of *A. indicum* L. (50 g)



Solvent extraction

Extracted with 500 ml of ethanol for 20 min at 37°C in a Soxhlet apparatus. It was then centrifuged. Supernatant collected and evaporated to dryness.

Active brown mass



Acid reflux

Refluxed with 70 ml of 1(N) HCL for 5 min on a water bath at 100 °C. It was then cooled and centrifuged. Supernatant was evaporated to dryness.

Active brown mass



Treatment with chloroform

Treated with 100 ml chloroform on a rotary shaker for 10 min. It was then centrifuged. Supernatant was evaporated to dryness.

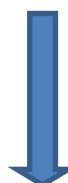
Active brown mass



Alumina column chromatography

Extracted with 20 ml of methanol for 10 min. It was then filtered. With filtrate alumina column chromatography was performed. Elution was done by isopropanol, chloroform mixture (50:50 v/v).

Third band was found active



Polyamide column chromatography

Eluent of active third band was evaporated to dryness. The dry mass was extracted with 20 ml ethanol for 10 min. It was then filtered. With filtrate polyamide column chromatography was performed. Elution was done by isopropanol, chloroform mixture (50:50 v/v).

Fourth band was active



Silica gel g column chromatography

Eluent of active fourth band was evaporated to dryness. The dry mass was extracted with 20 ml ethanol for 10 min. It was then filtered and the filtrate was subjected to silica gel column chromatography using silica gel G as adsorbent. Elution was done by isopropanol, chloroform mixture (50:50 v/v).

Second band was found active



Crystallization

Eluent of the active second band obtained from the above step was evaporated to dryness. Repeated crystallization was done from Benzene : ethyl acetate (60:40, v/v) mixture.

Crystals obtained (6.2 mg)

2.5 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.*^[8] with slight modifications. 400 µl of 0.1 M sodium phosphate buffer (pH 7.0), 500 µl of 1% starch solution, isolated compound (10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml) separately dissolved in DMSO and 50 µl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml) were mixed and incubated at 37 °C for 10 min. 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent was then added. The mixture was kept in a boiling water bath for 5 min and then diluted with 20 ml of distilled water. The absorbance was recorded at 540 nm. Control sample was prepared accordingly without test drug and acted as a negative control. Acarbose was used as positive control. Inhibition capacity of test drug and acarbose were calculated as following:

$$\text{Inhibition Percentage (\%)} = 1 - \frac{\text{DO sample}}{\text{DO control}} \times 100.$$

All tests were done for five sample replications. IC₅₀ value which is the concentration required to inhibit 50% of alpha amylase activity was calculated in each case.

Table -1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and of the isolated compound from *A. indicum* L.

Drug/solvent extract	Concentration (µg/ml)	% of inhibition	IC ₅₀ Value (µg/ml)
Acarbose	10	21.8±1.0	66.5±1.0
	20	27.5±1.0	
	40	34.8±1.1	
	60	46.2±1.4	
	80	57.9±1.2	
	100	60.8±1.6	
Isolated compound from <i>A. indicum</i> L.	10	25.1±0.9	62.3±1.0
	20	33.4±1.1	
	40	40.8±1.1	
	60	48.4±1.5	
	80	60.7±1.4	
	100	72.1±1.5	

Values are mean ± SE

A. indicum L. leaves in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml, however, showed 25.1±0.9, 33.4±1.1, 40.8±1.1, 48.4±1.5, 60.7±1.4, 72.1±1.5 percent of inhibitions in alpha amylase activity respectively with IC₅₀ value 62.3±1.0 µg/ml.

4. DISCUSSION

A. indicum (Family: *Malvaceae*), commonly known as Abutilon, Indian mallow is found in Sri Lanka, topical regions of America and Malaysia. The plant is a perennial shrub, softly tomentose and up to 3 m in height. Stems are stout and branched, root is cylindrical, bark is flattened and the seeds are minutely stellate-hairy, black or dark brown.^[10-11]

The plant has several traditional uses. In Chinese medicine seeds are used as an emollient and demulcent.

2.6 Statistical calculation

This was done by SPSS 20. The statistical significance of enzyme inhibitions between test drugs and acarbose, the known inhibitor, was evaluated with Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.^[9]

3. RESULTS

3.1 Isolation of compound

One compound was isolated from leaves of *A. indicum* L.

3.2 Alpha amylase inhibition activity of the isolated compound

Results are summarized in Table -1.

Acarbose, standard alpha amylase inhibitor, in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 21.8±1.0, 27.5±1.0, 34.8±1.1, 46.2±1.4, 57.9±1.2, 60.8±1.6 percent of inhibitions in alpha amylase activity respectively with IC₅₀ value 66.5±1.0 µg/ml. Isolated compound from

In Unani systems of medicine the plant is used in chest troubles, piles, bronchitis and gonorrhoea. Folk practitioners use this plant as mouthwash, for curing allergy, blood dysentery and fever^[12].

Many bioactive compounds have isolated from different parts of the plant. Few of them are, caffeic acid, fumaric acid, (R)-N-(1'-methoxycarbonyl-2'phenylethyl)-4-hydroxybenzamide, phydroxybenzoic, galacturonic, p-β-D-glycosyloxybenzoic β-sitosterol, vanillic acid, p-coumaric acid, abutilon A, farnesol, borenol, 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol, luteolin 7-O-beta glucopyranoside, chrysoeriol 7-O-betaglucopyranoside, quercetin 3-O-alpha-rhamnopyranosyl (1 --> 6)-beta-glucopyranoside geraniol, geranyl acetate, elemene and α-cineole, vanillic acid, caffeic acid, p-hydroxybenzoic

acid, β – sitosterol, fumaric acid, p-coumarin, p - β – Dglucosyloxybenzoic acids, gluco-vanilloyl glucose, amino acids like threonine, serine, leucine, aspartic acid, histidine etc.^[13-14]

A. indicum has several pharmacological activities such as anti-inflammatory, anti-proliferative, anti-arthritic, anti-diabetic, anti-pyretic, anti-malarial, anti-oxidant, anti-microbial, anti-fertility, anti-cancer, anti-diarrhoeal, anti-convulsant, anti- asthmatic, anti –ulcer, anti-bacterial, anti-estrogenic, hepatoprotective, hypoglycemic, wound healing etc.^[15-16] Alpha amylase inhibitory activity of root and leaves of *A. indicum* L. is known in literature.^[17-18]

Recently we have shown that ethanol extract of *A. indicum* L. leaves has maximum *in vitro* alpha amylase inhibitory activity. We, therefore, intended to isolate alpha amylase inhibitor from *A. indicum* L. leaves. Adopting standard techniques of isolation we isolated one compound from the plant leaves. The compound showed alpha amylase inhibitory activity which was comparable to that of acarbose, the standard alpha amylase inhibitor, both in terms of concentrations (Figure – 1) and IC₅₀ Value (Figure – 2).

Alpha amylase inhibitors are being isolated from various sources by different workers^[19]. In the present work we have isolated an alpha amylase inhibitor from *A. indicum* leaves. The compound now needs characterization. Process of characterization by different spectrometric techniques is now going on in our laboratory.

5. CONCLUSION

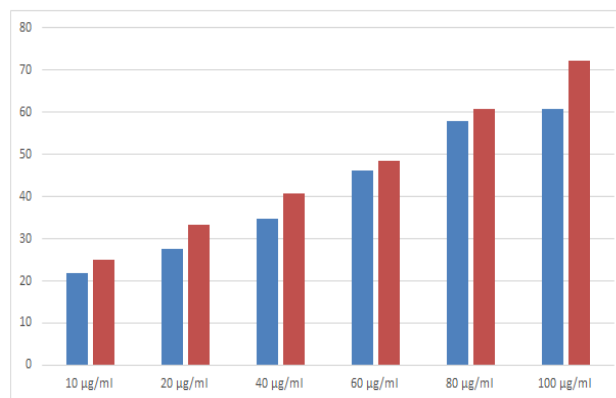
One of the therapeutic strategies of Type -2 diabetes mellitus is to keep postprandial blood glucose of the patients under control. These can be done applying by alpha amylase inhibitors.

By inhibiting splitting of complex carbohydrate into free sugars alpha amylase inhibitors keep postprandial blood glucose level within normal range. In the present work the compound which was isolated from *A. indicum* L. leaves showed strong alpha amylase inhibitory activity. The compound, therefore, may be included in future in the list of drug for Type – 2 diabetes mellitus.

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Conflict of interest: The authors declare that they have no conflict of interest.



■ Acarbose ■ Compound isolated from *A. indicum* L. leaves

Figure – 1: Alpha amylase inhibitory activity in different doses of acarbose and in the same doses of the compound isolated from *A. indicum* L. leaves.

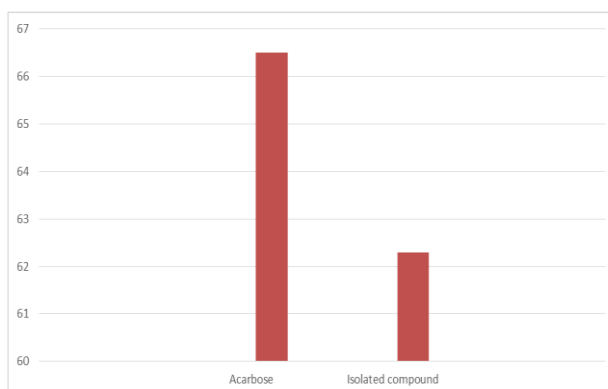


Figure – 2: IC₅₀ values (µg/ml) in alpha amylase inhibitory activity of acarbose and the compound isolated from *A. indicum* L. leaves.

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