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IN VITRO STUDY ON ALPHA AMYLASE INHIBITORY ACTIVITY OF *ABUTILON INDICUM*: EFFECT OF EXTRACTION SOLVENTS

Tanaya Ghosh and Prasanta Kumar Mitra*

Department of Medical Biotechnology, Sikkim Manipal University, Sikkim ManipalInstitute of Medical Sciences, Gangtok, Sikkim, India.

***Corresponding Author: Prasanta Kumar Mitra**
Department of Medical Biotechnology, Sikkim Manipal University, Sikkim ManipalInstitute of Medical Sciences, Gangtok, Sikkim, India.

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ABSTRACT
Abutilon indicum Linn. (*A. indicum* L.), a medicinal plant, is used in traditional medicine for treatment of toothache, catarrhal bilious, bronchitis, diarrhoea, gonorrhoea and inflammation of bladder as well as in fever. The plant has wide pharmacological activities too. The plant also possesses alpha amylase inhibitory activity. Aim of the present work was to see effect of solvent extracts on *in vitro* alpha amylase inhibitory activity of *A. indicum* L. leaves. *A. indicum* L. leaves were collected from medicinal plant garden of North Bengal University and identified by the taxonomist. Solvent extractions of the leaves were made separately by using chloroform, petroleum ether, ethanol, isopropanol and hexane. Extracts were separately dried and processed for *in vitro* alpha amylase inhibitory activity by standard method. Acarbose, an alpha amylase inhibitor, was used as control. Results showed that ethanol extract of *A. indicum* L. leaves had maximum alpha amylase inhibitory activity in comparison to that of other solvent extracts. This study therefore indicates that ethanol extract of *A. indicum* L. leaves may be used in the management of diabetes.

KEYWORDS: *Abutilon indicum* linn. leaves; solvent extractions; alpha amylase inhibitory activity, acarbose, diabetes.

1. INTRODUCTION
*A. indicum (*Family: *Malvaceae),* commonly known as Abutilon, Indian mallow is found in Sri Lanka, topical regions of America and Malaysia. It is also found as a weed in sub-Himalayan tracts, hills up to 1200 m and in hotter parts of India. The plant is a perennial shrub, softly tomentose and up to 3 m in height. Stems are stout and branched, root is cylindrical, flowers are P. 1yellow or orange-yellow. Leaves are evergreen, stipulate, and cordate. Fruits are capsule, denselypubescent. The bark is flattened and the seeds are minutely stellate-hairy, black or dark brown.[1,2]

The plant has several traditional uses. Whole plant is used as a febrifuge, anthelminitic. It is also used in gout, ulcers, inflammation and in treatment of urinary tract problems. Seeds are used in piles as laxative. Bark is given in strangury and urinary complaints and is valued as a diuretic. Decoction of leaves is used in toothache, catarrhal bilious, bronchitis, diarrhoea, gonorrhoea and inflammation of bladder as well as in fever. Infusion of root is useful in fever as a cooling medicine, heamatouria, strangury and also in leprosy. Root is also used as a pulmonary sedative and diuretic. In Chinese medicine seeds are used as an emollient and demulcent. In Unani systems of medicine the plant is used in chest troubles, piles, bronchitis and gonorrhea. Folk practitioners use this plant as mouthwash, for curing allergy, blood dysentery and fever.[3]

Many bioactive compounds have isolated from different parts of the plant. Whole plant contains caffeic acid, fumaric acid, *β*-sitosterol, vanillic acid, *p*-coumaric acid, abutilon A, (R)-N-(1'-methoxycarbonyl-2'phenylethyl)-4-hydroxybenzamide, phydroxybenzoic, galacturonic, *p*-β-D-glycosyloxybenzoic etc. Oil of the plant consists of farnesol, borenol, geraniol, geranyl acetate, elemene and α-cineole. Aerial Part contains 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. Leaves contain terpenes, hydrocarbon, flavonoids, amino acids, ketone, aldehyde, fatty acids like stearic, palmitic linoleic, oleic etc. Root contains endesmol,α-pinene, caryophyllene, caryophyllene oxide. Flower contains flavonoids like apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol, luteolin 7-O-beta glucopyranoside, chrysoeriol 7-O-betaglucopyranoside, quercetin 3-Oalpha- rhamnopyranosyl (1 --> 6)-beta-glucopyranoside. Fruits contain flavonoids and alkaloids Seed contains water soluble galactomannan, *cis* 12, 13-

epoxyoleic (vernolic) acid, 9, 10- methylene octadec-9-enoic (sterculic) acid, as well as 8, 9- methylene-heptadec-8-enoic (malvalic) acid.[4,5]

A. indicum has several pharmacological activities like anti-oxidant, anti-microbial, anti-fertility, anti-cancer, anti-diarrhoeal, anti-convulsant, anti- asthmatic, anti – ulcer, anti-bacterial, anti- P. 2 inflammatory, anti-proliferative, anti-arthritic, anti-diabetic, anti-pyretic, anti-malarial, anti-estrogenic, hepatoprotective, hypoglycemic and wound healing. Further, the plant showed analgesic and sedative property as well as larvicidal – diuretic - immunomodulatory activity. [6,7]
Alpha amylase inhibitory activity of root and leaves of *A. indicum* L. is known in literature.[8-9] Aim of the present work was to see effect of solvent extracts on in vitro alpha amylase inhibitory activity of *A. indicum* L. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

A. indicum L. leaves were collected from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India. 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.

***Abutilon indicum* Linn.**

2.2 Preparation of plant materials

Leaves of *A. indicum* L. were washed thoroughly, shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use. P. 3

2.3 Solvent extraction

Test drug (100 g) was extracted separately with 500 ml of chloroform, petroleum ether, ethanol, isopropanol and hexane in soxhlet at 37^{0}C for 15 minutes. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. This was applied separately for all extracts. Brown masses obtained were used for *in vitro* alpha amylase inhibition assay.

2.4 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.*[10] with slight modifications. 400 µl of 0.1 M sodium phosphate buffer (pH 7.0), 500 µl of 1% starch solution, 10 µg/ml , 20 µg/ml , 40 µg/ml, 60 µg/ml , 80 µg/ml and 100 µg/ml of all extracts 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 capacities of test drug and acarbose were calculated as following:

Inhibition Percentage (%) = 1 − DO sample/DO control × 100.

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

2.5 Statistical calculation

This was done by SPSS 20. The statistical significance of enzyme inhibitions between test drugs and acarbose, the known inhibitor of alpha amylase, was evaluated with Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.[11]

3. RESULTS

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 20.17±1.0, 22.21±1.1, 34.44±1.1, P. 4

Table -1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and different solvent extracts of *A. indicum* L. leaves.

Drug/solvent extract	Concentration (µg/ml)	% of inhibition	IC_{50}Value (µg/ml)
Acarbose	10	20.17±1.0	67.96±1.3
	20	22.21±1.1	
	40	34.44±1.1	
	60	44.85±1.2	
	80	57.22±1.5	
	100	60.65±1.7	
Chloroform extract of *A. indicum* L. leaves	10	12.21±0.1	71.57±1.4
	20	18.22±1.0	
	40	36.62±1.1	
	60	42.51±1.1	
	80	54.66±1.5	
	100	64.55±1.7	
Petroleum ether extract of *A. indicum* L. leaves	10	21.14±0.8	58.12±1.3
	20	24.26±1.1	
	40	41.32±1.1	
	60	52.17±1.4	
	80	58.33±1.5	
	100	71.54±2.0	
Ethanol extract of *A. indicum* L. leaves	10	27.11±1.0	38.34±1.1 *
	20	38.22±1.1	
	40	66.55±1.4	
	60	70.21±1.5	
	80	74.25±2.0	
	100	84.22±2.0	
Isopropanol extract of *A. indicum* L. leaves	10	22.11±1.0	51.65±1.1
	20	28.23±1.1	
	40	46.23±1.2	
	60	57.51±1.4	
	80	64.66±1.6	
	100	77.37±1.8	
Hexane extract of *A. indicum* L. leaves	10	14.81±1.0	62.07±1.2
	20	23.23±1.0	
	40	41.43±1.1	
	60	48.36±1.3	
	80	56.25±1.4	
	100	68.11±1.5	

Values are mean ± SE *Significant

44.85±1.2, 57.22±1.5, 60.65±1.7 respectively percent of inhibitions in alpha amylase activity with IC_{50} value 67.96±1.3 µg/ml. Chloroform extract of *A. indicum* L. leaves, on the other hand, P. 5 showed 12.21±0.1, 18.22±1.0, 36.62±1.1, 42.51±1.1, 54.66±1.5, 64.55±1.7 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC_{50} value came 71.57±1.4 µg/ml.

Petroleum ether extract of *A. indicum* L. leaves in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 21.14±0.8, 24.26±1.1, 41.32±1.1, 52.17±1.4, 58.33±1.5,71.54±2.0percent of inhibitions in alpha amylase activity respectively with IC_{50} value 58.12±1.3 µg/ml.

Ethanol extract of *A. indicum* L. leaves, however, showed 27.11±1.0, 38.22±1.1, 66.55±1.4, 70.21±1.5, 74.25±2.0, 84.22±2.0 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100µg/ml respectively. IC_{50} value was 38.34±1.10 µg/ml.

Isopropanol extract of *A. indicum* L. leaves in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 22.11±1.0, 28.23±1.1,46.23±1.2, 57.51±1.4, 64.66±1.6, 77.37±1.8 percent of inhibitions in alpha amylase activity respectively with IC_{50} value 51.65±1.1 µg/ml.

Hexane extract of *A. indicum* L. leaves, on the other hand, showed 14.81±1.0, 23.23±1.0, 41.43±1.1, 48.36±1.3, 56.25±1.4, 68.11±1.5 percent of inhibitions respectively in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml. IC_{50} value came 62.07±1.2 µg/ml.

4. DISCUSSION

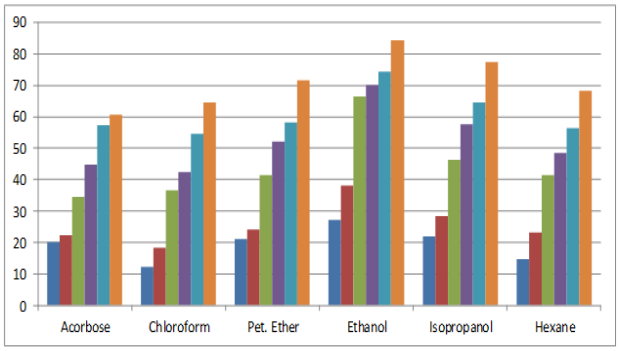
According to World Health Organization (WHO), diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia (high levels of glucose in blood) with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Insulin is the hormone releases from beta cells of pancreas and controls blood glucose levels by signaling liver, muscle and fat cells to take glucose from blood to generate energy.[12,13]

Diabetes mellitus is mainly of two types. P. 6

Type 1- Insulin dependent diabetes mellitus (IDDM) also referred to as juvenile diabetes and known to affect only 5% of the diabetic population. In this case body makes little or even no insulin and the patient therefore requires insulin daily to survive. Type 2- Non-insulin dependent diabetes mellitus (NIDDM) usually develops in adults over the age of 40. In this case body is incapable of responding to insulin. However, diabetes may develop during pregnancy and, if so, it is known as gestational diabetes.[14,15]

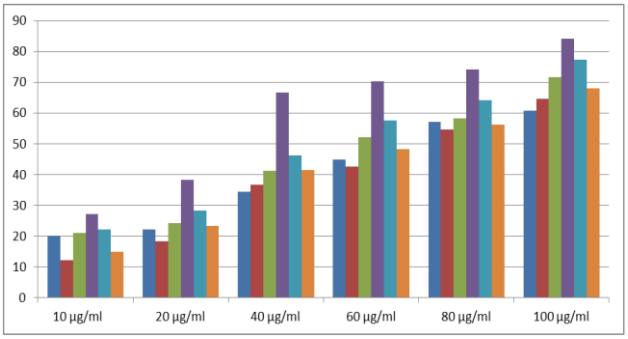
Diabetes mellitus particularly Type – 2 diabetes mellitus is characterized by postprandial hyperglycemia. One of the therapeutic approaches, therefore, is to reduce postprandial hyperglycemia.[16] This can be done by inhibiting carbohydrate splitting enzymes. One such enzyme is alpha amylase which hydrolyses complex carbohydrates of food to free sugars. Inhibition of alpha amylase reduces hydrolysis of complex carbohydrate thereby postprandial hyperglycemia is checked.[17] Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of Type - 2 diabetes mellitus.[18] In this context medicinal plants were also investigated for alpha amylase inhibitory activity and many plants were found having alpha amylase inhibitory activity.[19]

The present work showed alpha amylase inhibitory activity of *A. indicum* L. leaves in *in vitro* experiments. Ethanol, isopropanol, petroleum ether, hexane and chloroform extracts of *A. indicum* L. leaves in all concentrations exerted *in vitro* alpha amylase inhibitory activity which was comparable to that of acarbose, standard alpha amylase inhibitor (Figure – 1). Maximum activity, however, was noted in ethanol extract. This is evident when examined alpha amylase inhibitory activity in all doses (10, 20, 40, 60, 80 and 100 µg/ml) of ethanol extract and the same doses of acarbose as well as chloroform, petroleum ether, isopropanol and hexane extracts (Figure – 2). This is further evident when examined IC_{50} value in alpha amylase inhibitory activity of ethanol extract of *A. indicum* L. leaves (38.34±1.10 µg/ml) and the IC_{50} values of acarbose (67.96±1.3 µg/ml), isopropanol (51.65±1.1 µg/ml), petroleum ether (58.12±1.3 µg/ml), hexane (62.07±1.2 µg/ml) and chloroform (71.57±1.4 µg/ml) extracts of *A. indicum* L. leaves (Figure – 3). P. 7



■ 10 µg/ml ■ 20 µg/ml ■ 40 µg/ml ■ 60 µg/ml ■ 80 µg/ml ■ 100 µg/ml

Figure – 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and different solvent extracts of *A. indicum* L. leaves.



■ Acarobose ■ Chloroform ■ Petroleum ether ■ Ethanol ■ Isopropanol ■ Hexane P. 8

Figure – 2: Alpha amylase inhibitory activity in different doses of acarbose and various solvent extracts of *A. indicum* L. leaves in the same doses.

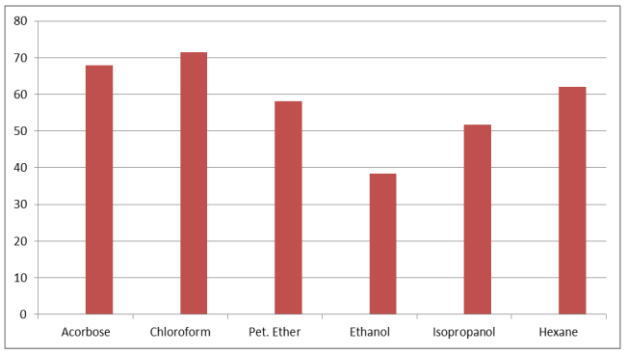


Figure – 3: IC_{50} values (µg/ml) in alpha amylase inhibitory activity of acarbose and different solvent extracts of *A. indicum* L. leaves.

Pant *et al.* observed that methanol extract of *A. indicum* L.leaves had maximum inhibition (%) on alpha amylase activity[8] while Florencio *et al.* noted that ethanol extract from *A. indicum* roots had maximum inhibition (%) on alpha amylase activity.[9] The present study, however, advocates use of ethanol extract of *A. indicum* L. leaves in Type – 2 diabetes mellitus to keep postprandial blood sugar level under control.

It is known that biological activity of medicinal plants depends on season.[20] We are now working on seasonal variation in alpha amylase inhibitory activity of *A. indicum* L. leaves.

5. CONCLUSION

Based on the present work compound responsible for alpha amylase inhibitory activity may be isolated from the ethanol extract of *A. indicum* L. leaves which, in turn, may be used in future as drug for diabetes.

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Conflict of interest: The authors declare that they have no conflict of interest. P. 9

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