



SEASONAL EFFECT ON *IN VITRO* ALPHA AMYLASE INHIBITORY ACTIVITY OF *ABUTILON INDICUM* LEAVES

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ABSTRACT

Abutilon indicum Linn. (*A. indicum* L.) is a medicinal plant being used in traditional medicine since long. Recently we have confirmed *in vitro* alpha amylase inhibitory activity of *A. indicum* L. leaves. As pharmacological activities of medicinal plants vary with season, aim of the present work was to see the seasonal effect on *in vitro* alpha amylase inhibitory activity of *A. indicum* L. leaves, if any. Leaves of *A. indicum* L. were collected in winter, summer, autumn and rainy seasons from medicinal plant garden of the North Bengal University and authenticated by the taxonomist. Leaves were extracted with ethanol and the extract was checked for *in vitro* alpha amylase inhibitory activity by the standard method. Acarbose, a standard alpha amylase inhibitor, was used as control. Results showed that *A. indicum* L. leaves of rainy season (June – August) had maximum alpha amylase inhibitory activity. Result was comparable to that of acarbose. Leaves of *A. indicum* L. of rainy season may therefore be utilized to keep post prandial blood sugar of the diabetic patient under control and thereby used in the treatment of diabetes.

KEYWORDS: *Abutilon indicum* Linn. Leaves, alpha amylase inhibitory activity, seasonal variation, acarbose, diabetes.

1. INTRODUCTION

Biological activities of plants vary with seasons of the year. Qinxue *et al.* studied seasonal variations in the antioxidant activity of ground bamboo *Sasa argenteostriatus* Leaves. They noted that the highest antioxidant activity appeared in December and the lowest was in May.^[1] Effect of seasonal variation on the antineoplastic activity of *Alstonias cholaris* R. Br. in HeLa cells was studied by Jagetia and Baliga. Highest cell killing effect was observed by the plant of summer collection.^[2] Osadebe *et al.* worked on seasonal variation for the antidiabetic activity of methanolic extract of *Loranthus micranthus* and noted that the activity is highest at the peak of the rainy season.^[3] Ncube *et al.* studied seasonal variation in antimicrobial activity of frequently used medicinal bulbous plants from South Africa and noted that the activity was higher in spring and winter than in other seasons.^[4] Effect of seasonal variation on the anti-inflammatory activity of *Sargassum wightii* was studied by Dar and coworkers. They found that the plant collected during winter was most effective in reducing carrageenan-induced edema in rats.^[5] Report from our laboratory showed that *Cassia alata* leaves during the period of May – June had maximum protective effect on anti tubercular drugs induced hepatotoxicity in rats.^[6] We also reported that UV

absorption property of *Amaranthus spinosus* was maximum during autumn in comparison to other seasons of the year.^[7]

A. indicum Linn. L. (Family: *Malvaceae*), a medicinal plant, commonly known as Abutilon, Indian mallow is being used in traditional medicine in the treatments of gout, ulcers, inflammation, since long.^[8] The plant has different pharmacological activities like anti-oxidant, anti-microbial, anti-fertility, anti-cancer, anti-diarrhoeal, anti-convulsant, anti-asthmatic, anti-ulcer, anti-bacterial etc.^[9,10] Recently we have confirmed that ethanol extract of *A. indicum* L. leaves could exert strong *in vitro* alpha amylase inhibitory activity. Paper is under communication. Aim of the present work was to see the seasonal effect on *in vitro* alpha amylase inhibitory activity of *A. indicum* L. leaves, if any.

2. MATERIAL AND METHODS

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 summer (March – May), rainy season (June – August), Autumn (September – November) and Winter (December – February). 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 powdered. The powder, used as test drug, was stored desiccated at 4 °C until further use.

2.3 Solvent extraction

Test drug (50 g) was extracted separately with 500 ml of ethanol in soxhlet at 37°C for 20 minutes. Ethanol was chosen as solvent as in our earlier study we have found that ethanol extract of *A. indicum* L. leaves had maximum *in vitro* alpha amylase inhibitory activity. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 °C. Brown mass obtained was 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.*^[11] 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 capacity of test drug and Acarbose were calculated as following:

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.

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 activity, was evaluated with Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.^[12]

3. RESULTS

Results are summarized in Table -1. Acarbose, standard alpha amylase inhibitor, had 21.3±1.0, 24.1±1.1, 36.3±1.1, 46.5±1.5, 58.1±1.4, 60.6±1.2 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC₅₀ value was 63.4±1.2 µg/ml.

Ethanol extract of *A. indicum* L. leaves of Summer (March – May) showed 10.1±0.7, 16.5±1.0, 35.3±1.2, 40.5±1.4, 46.6±1.5, 61.3±1.7 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC₅₀ value came 73.2±1.4 µg/ml.

Ethanol extract of *A. indicum* L. leaves during rainy season (June – August) in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed percent of inhibitions in alpha amylase activity as 23.2±0.9, 31.5±1.1, 47.3±1.4, 56.1±1.3, 60.5±1.5, 70.8±2.0 respectively with IC₅₀ value 54.4±1.1 µg/ml.

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

Drug/solvent extract	Concentration (µg/ml)	% of inhibition	IC ₅₀ Value (µg/ml)
Acarbose	10	21.3±1.0	63.4±1.2
	20	24.1±1.1	
	40	36.3±1.1	
	60	46.5±1.5	
	80	58.1±1.4	
	100	60.6±1.2	
Ethanol extract of <i>A. indicum</i> L. leaves Summer (March – May)	10	10.1±0.7	73.2±1.4
	20	16.5±1.0	
	40	35.3±1.2	
	60	40.5±1.4	
	80	46.6±1.5	
	100	61.3±1.7	
Ethanol extract of <i>A. indicum</i> L. leaves Rainy season (June – August)	10	23.2±0.9	54.4±1.1*
	20	31.5±1.1	
	40	47.3±1.4	
	60	56.1±1.3	
	80	60.5±1.5	
	100	70.8±2.0	
Ethanol extract of <i>A. indicum</i> L. leaves Autumn (September – November)	10	17.9±1.0	59.5±1.7
	20	22.2±1.1	
	40	41.3±1.5	
	60	50.7±1.4	
	80	53.7±1.1	
	100	61.7±2.0	
Ethanol extract of <i>A. indicum</i> L. leaves Winter (December – February)	10	10.1±1.1	79.1±1.7
	20	13.9±1.1	
	40	31.1±1.3	
	60	37.5±1.5	
	80	51.2±1.5	
	100	57.5±1.6	

Values are mean ± SE *Significant

Ethanol extract of *A. indicum* L. leaves of Autumn (September – November) had 17.9±1.0, 22.2±1.1, 41.3±1.5, 50.7±1.4, 53.7±1.1, 61.7±2.0 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC₅₀ value found, 59.5±1.7 µg/ml.

Ethanol extract of *A. indicum* L. leaves of Winter (December – February) had 10.1±1.1, 13.9±1.1, 31.1±1.3, 37.5±1.5, 51.2±1.5, 57.5±1.6 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC₅₀ value came, 79.1±1.7 µg/ml.

4. DISCUSSION

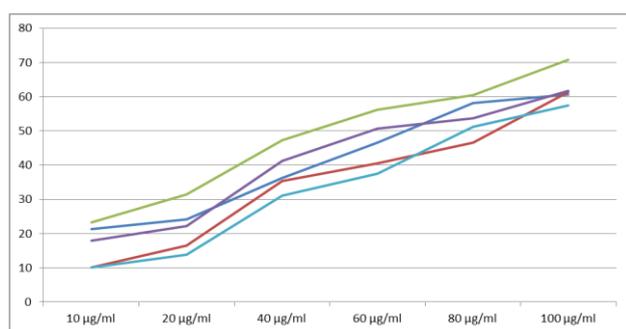
Diabetes mellitus has characteristic symptoms like thirst, polyuria, blurred vision and loss of weight resulting long-term damage, dysfunction and failure of various organs such as, eyes, kidneys, nerves, heart, liver and veins. Disturbance in these organs can lead to death. An estimated 1.6 million deaths were directly caused by diabetes globally in 2016 and nearly 1 million Indians die due to diabetes every year. One of the therapeutic approaches of diabetes mellitus specially type – 2

diabetes mellitus is to reduce post prandial blood glucose level of the patient.^[13] This may be done by several ways. One way is to use inhibitors of those enzymes which are responsible for the hydrolysis of complex carbohydrates of food to free sugars. Alpha amylase is one such enzyme which hydrolyses complex carbohydrates of food to free sugars.^[14] Therefore, by inhibiting activity of alpha amylase post prandial blood glucose level may be kept under control. Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of type - 2 diabetes mellitus.^[15] Still continuous effect is going on in search of alpha amylase inhibitors from different sources which even extended in the field of medicinal plants. Now-a-days, many medicinal plants are known possessing alpha amylase inhibitory activity.^[16]

Since long in traditional medicine leaves of *A. indicum* L., a weed in sub-Himalayan tracts, is being used in the treatment for diabetes.^[17-18] Recently we found that *A. indicum* L. leaves of this region exert alpha amylase inhibitory activity. Since activity of medicinal plant varies with season.^[1-7] we undertook the present study to

note seasonal variation in alpha amylase inhibitory activity of *A. indicum* L. leaves, if any.

The present work showed that *A. indicum* L. leaves of summer, winter, autumn and rainy seasons in all concentrations (10, 20, 40, 60, 80 and 100 $\mu\text{g/ml}$) exerted alpha amylase inhibitory activity like acarbose, one standard alpha amylase inhibitor in *in vitro* experiments (Figure-1). Activity was dose dependent and comparable to that of acarbose (Figure – 2). Maximum activity, however, was noted in *A. indicum* L. leaves of rainy season when compared with the alpha amylase inhibitory activity of the leaves of other seasons and with acarbose (Figure – 3). This is further evident when examined IC_{50} value in alpha amylase inhibitory activity of the plant leaves of different seasons (Figure – 4).



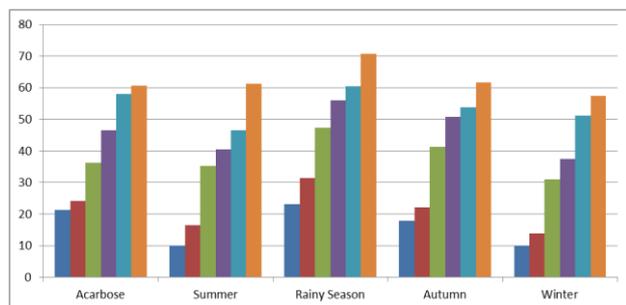
Acarbose

A. indicum L. leaves of

Summer

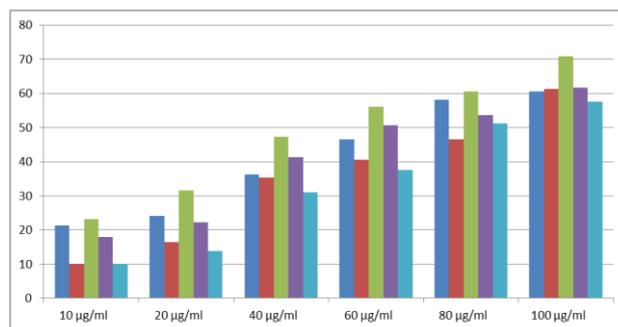
Autumn

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



■ 10 $\mu\text{g/ml}$ ■ 20 $\mu\text{g/ml}$ ■ 40 $\mu\text{g/ml}$ ■ 60 $\mu\text{g/ml}$ ■ 80 $\mu\text{g/ml}$ ■ 100 $\mu\text{g/ml}$

Figure – 2: Dose dependent alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and ethanol extracts of *A. indicum* L. leaves of different seasons.



■ Acarbose ■ Summer ■ Rainy season
■ Autumn ■ Winter

Figure – 3: Comparison of alpha amylase inhibitory activity of acarbose and ethanol extracts of *A. indicum* L. leaves of different seasons in varying concentrations.

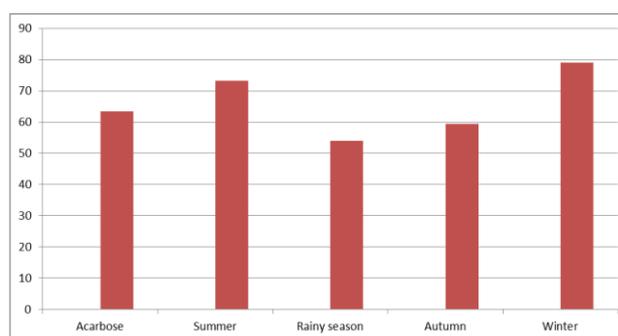


Figure – 4: IC_{50} values ($\mu\text{g/ml}$) in alpha amylase inhibitory activity of acarbose and ethanol extracts of *A. indicum* L. leaves of different seasons.

Isolation of alpha amylase inhibitor from *A. indicum* L. leaves of rainy season is now needed. Presently work is going on in our laboratory in this direction.

5. CONCLUSION

Diabetes mellitus is increasing rapidly. There were approximately 108 million diabetic patients in the world in 1980 but in 2014 the number has been increased to 422 million. Due to this increase diabetes took several lives till today. Under the circumstances the present work throws some light of getting alpha amylase inhibitor from *A. indicum* L. leaves which may be used in future as a drug for Type-2 diabetes mellitus.

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

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