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EVALUATION OF ALPHA-AMYLASE INHIBITORY ACTIVITY OF *GREWIA ABUTILIFOLIA* LEAVES

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ABSTRACT

Alpha-amylase inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates (such as starch and table sugar).Inhibition of Alpha-amylase enzyme that plays a role in digestion of starch and glycogen is considered a strategy for the treatment of disorders in carbohydrate uptake such as diabetes and obesity.Plants are an important source of chemical constituents with potential for inhibition of Alpha-amylase and can be used as therapeutic or functional food sources. *Grewia abutilifolia* belonging to the family Tiliaceae,is a tree and that is used in traditional medicine for the treatment of diabetic mellitus. In the present study, methanolic extracts of *Grewia abutilifolia* leaves which are used in the Ayurvedic traditional system of medicine to treat diabetes were tested for their inhibitory effect on α-amylase. The crude methanol extracts (6.88 ± 0.14) of *Greiwa abutilifilia* with pet-ether fraction, (21.13 ± 25.24), chloroform fraction(6.02 ± 1.15) and methanol+water fraction (5.2± 0.80). Among all fractions acarbose & methanol+water showed highest percentage inhibition & the Ic_{50} value was 5.7 ± 0.24 & 5.2 ± 0.80. So, methanol + water extracts has the highest percentage of inhibition than acarbose. As a results, *Grewia abutilifolia* leaves properly inhibits the alpha-amylase in diabetes.

KEYWORDS: Alpha-amylase, Carbohydrate Metabolism, Diabetes Mellitus, *Grewia abutilifolia*.

INTRODUCTION

Now a days, the dependency of the medicine on plants materials is becoming clearer. However, this dependency upon medicinal plants is still not widely acknowledged.[1] Plants possess a large number of vital compounds that might form a part of healthy diet. Traditionally, different parts of plants were used directly as pharmaceuticals.[2] Clinically useful chemicals are now being obtained from plants even those that have not been classified before as medicinal plants . One of the strategies and methods adopted to cure diabetes mellitus involves the inhibition of carbohydrate digesting enzymes such as amylase.[3-4] Alpha-amylase inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates (such as starch and table sugar).[5]Carbohydrates are normally converted into simple sugars (monosaccharides) which can be absorbed through the intestine.[6] Hence, alpha-glucosidase inhibitors reduce the impact of carbohydrates on blood sugar.[7]Thye are used to establish greater glycemic control over hyperglycemia in diabetes mellitus type 2, particularly with regard to postprandial hyperglycemia.[8] They may be used as monotherapy in conjunction with an appropriate diabetic diet and exercise or they may be used in conjunction with other anti-diabetic drugs.[9] *Grewia Abutilifolia* plant leaves are found in Bangladesh.[10-13] It is used as a remedy for acne and a root decoction as a remedy for fever.[14] The bark yields good quality fibre that is made into rope.

MATERIALS AND METHODS

Collection, Identification & authentication of plant

The plant material (leaves) was collected during the month of March, 2015 from Comilla Hill track, Bangladesh in fresh condition and identified by an expert taxonomist. A voucher specimen was submitted to the National Herbarium Bangladesh (accession number: 41884). Leaves were then washed properly to remove dirty materials and shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature for better grinding. The dried leaves were ground into coarse powder by a grinding machine in the department of Pharmacy, Southeast University.

Extraction of the plant materials: Powdered plant materials (leaves) having a weight of about 500 gm.were taken in an amber colored reagent bottle and soaked in 1.5 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary

evaporator under reduced pressure at 52°C temperature to afford crude extract (45.39 gm).

Solvent-solvent partitioning of crude extract (*Grewia Abutofilia*): An aliquot (20gm) of the concentrated methanol extract was fractionated by modified Kupchan method with petroleum ether, chloroform and water and the amount of fractions obtained is given in the following table:

Table. 1: Different fractions with amount of *Grewia abutilifolia*.

Name of fractions	Weight of fractions (gm)
Petroleum ether fraction	7.37
Chloroform fraction	7.25
Aqueous fraction	5.38

Alpha-amylase inhibition test

Principle

[15]Diabetes mellitus is a complex disease that is characterized by gross derangement in carbohydrate, protein, and fat metabolism.[16]It is a progressive metabolic disorder of glucose metabolism that eventually leads to micro and macro vascular changes causing secondary complications that are difficult to manage.[17]Type-1 diabetes results from inadequate synthesis of insulin by beta -cells of the pancreas while type II diabetes is characterized primarily by insulin resistance (a condition in which peripheral cells do not respond normally to insulin) or beta-cell dysfunction.[18]Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules.[19]On the other hand, mammalian alpha-glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet.[20] Inhibitors of alpha-amylase and alpha-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion.

Preparation of 0.5M Tris-Hcl buffer (^{pH} 6.9)

[21-22]Tris – Hcl was added with calcium chloride in 800 ml of water.Then added slowly 200ml of water.Then check the pH by the pH meter.If the pH was basic added acid & if the pH was acidic added base.

Preparation of starch solution

100mg starch was mixed with 10ml Tris Hcl buffer.

Preparation of α-amylase solution: 0.007gm α-amylase was dissolved in 100ml of Tris Hcl buffer.

Preparation of 50% acetic acid solution: 50gm / 50ml acetic acid was dissolved in 100ml of water.

To conduct this experiment,8 test tube were taken.[23]0.6 ml starch solution taken in each test tube Boiled at 90°C for 10 minute.Then pre incubate at 37°C for 5 minute.0.6ml of sample/standard solution was added to each test tube.[24]0.3ml of α-amylase solution is incubate at 37°Cfor 10 minutes.Then added 1.5ml of 50% acetic acid solution.Reaction mixture was centrifuged at 3000 rpm for 8 minutes.Then taken absorbance of supernatant at 595 nm.

RESULTS AND DISCUSSION

Alpha-amylase inhibition studies demonstrated that the extracts of *Grewia abutifolia* leaf had inhibitory activity. In all experiments, control samples were prepared accordingly without any plant extract and were compared with the test samples containing various concentrations of the plant extract. Acarbose was used as the standard.

Table. 02: Alpha-amylase inhibitor activity of different fractions of *Grewia Abutilifolia* at different concentrations.

Name of sample	Conc. (µg/ml)	% of inhibition			% of inhb. Mean	% of inhb. ± STD	IC_{50}
		A	b	c			(µg/ml)
CME	7.81	58.07	56.25	55.87	56.73	1.18	6.88 ± 0.14
	15.62	60.05	72.13	60.36	64.18	6.89	
	31.25	64.69	74.36	67.14	68.73	5.03	
	62.5	69.69	80.45	74.25	74.80	5.40	
	125	79.04	86.15	79.25	81.48	4.05	
	250	86.12	90.25	82.19	86.19	4.03	
Pt-Ether fraction	7.81	73.37	50.24	49.85	57.82	13.47	21.13 ± 25.24
	15.62	80.17	55.24	53.65	63.02	14.87	
	31.25	86.66	64.49	62.25	71.13	13.49	
	62.5	86.97	70.25	70.25	75.82	9.65	
	125	86.12	72.19	82.29	80.20	7.20	
	250	92.92	76.37	86.19	85.16	8.32	
Chloroform fraction	7.81	70.25	53.25	75.24	66.25	11.53	6.02 ± 1.15
	15.62	75.36	60.15	62.03	65.85	8.29	
	31.25	79.05	64.25	75.45	72.92	7.72	
	62.5	82.72	79.45	82.15	81.44	1.75	
	125	83.85	83.25	88.05	85.05	2.62	

	250	85.55	88.19	90.25	88.00	2.36	
Methanol + water fraction	7.81	90.93	70.25	67.45	76.21	12.82	5.2 ± 0.80
	15.62	91.76	74.02	70.25	78.68	11.49	
	31.25	92.06	81.5	76.15	83.24	8.10	
	62.5	92.35	89.15	89.25	90.25	1.82	
	125	93.48	90.25	86.12	89.95	3.69	
	250	95.75	94.48	87.25	92.49	4.59	

Table. 3: Alpha-amylase inhibitor activity of *Acarbose*(Standard)at different concentrations.

Name of sample	Conc. (µg/ml)	% of inhibition			% of inhb. Mean	% of inhb. ± STD	IC_{50} (µg/ml)
		a	b	c			
Acarbose (Standard)	7.81	70.27	65.27	69.87	68.47	2.78	5.7 ± 0.24
	15.62	71.15	66.15	61.45	66.25	4.85	
	31.25	81.32	76.32	70.32	75.99	5.51	
	62.5	83.67	78.67	75.67	79.34	4.04	
	125	93.34	88.34	83.25	88.31	5.05	
	250	95.57	90.57	85.37	90.50	5.10	

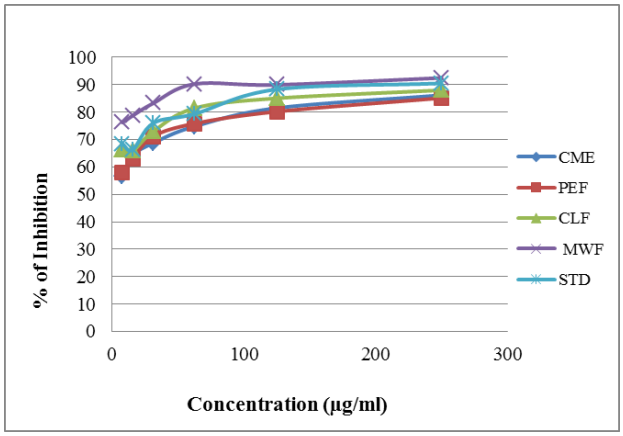

Figure. 1: Alpha-amylase inhibitor activity of CME, PEF, CLF, MWF & Acarbose(STD) of *Greiwa abutilifolia* at different concentrations.

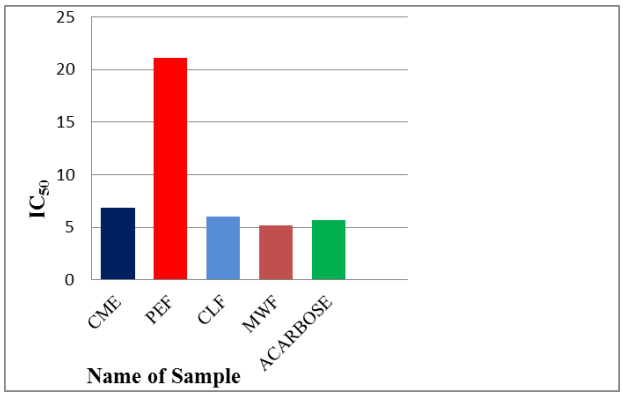

Figure. 2: IC_{50} (µg/ml) values of different extractives of *Grewia abutilifolia* for Alpha- amylase activity Assay.

DISCUSSION

[25]Alpha-amylase inhibitors are oral anti-diabetic drugs used for diabetes mellitus type 2 that work by preventing the digestion of carbohydrates (such as starch and table sugar). Carbohydrates are normally converted into simple sugars (monosaccharides) which can be absorbed through the intestine.[26-27] Acarbose is used as standard in this result which blocks pancreatic alpha-amylase in addition to inhibiting membrane-bound alpha-glucosidases.[28] Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine.[29] Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates.[30] Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules.[31-32] In diabetic patients,the short-term effect of these drugs therapies is to decrease current blood glucose levels,the long-term effect is a small reduction in hemoglobin $_{A1c}$ level.[33] In alpha amylase inhibition test the crud methanolic extract of *Grewia abutilifolia* with known antidiabetic activity was investigated for their potential to inhibit α-amylase activity.[34]Among the different fractions of the crude extract such as petroleum ether,chloroform and aqueous fractions,were examined at a different concentration (µg/ml).[35-36]Among all fractions acarbose & methanol + water showed highest percentage inhibition & the Ic_{50} value was 5.7 ± 0.24 & 5.2 ± 0.80.[37] So, methanol + water has the highest percentage of inhibition than acarbose. pet-ether showed lowest percentage of inhibition & the Ic_{50} value was 21.13 ± 25.24.Chloroform fraction & Crude Methanol extract showed moderate percentage of inhibition & the Ic_{50} value was 6.02 ± 1.15& 6.88 ± 0.14 respectively.[38] So, the crud methanol extract of *Grewia abutilifolia* showed alpha amylase inhibition activity.

CONCLUSION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and its type II is the major form of diabetes,accounting for 90% of cases worldwide.There are many and diverse therapeutic strategies in the management of Type II diabetes.The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels.The leaves and bark have been also used in medicated enema and used in traditional medicine for the treatment of diabetic mellitus

and is widely distributed all over the Bangladesh. In this research,*Grewia abutilifolia* are used for alpha amylase inhibition test. The result demonstrates that, In alpha amylase inhibition test among all fractions of *Grewia abutilifolia* acarbose & methanol + water showed highest inhibition respectively. Further studies needed to determine active compounds responsible for these activities and I think that, this project work will help to identify the active constituents, structure and their action mechanism responsible for the activity and this project will also help a new investigator to proceed the future research.

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