Available online on 10.01.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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In-vitro studies on inhibition of alpha amylase and alpha glucosidase by plant extracts of alternanthera *Pungens kunth*

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ABSTRACT

The aim of this work was to evaluate the alpha amylase and alpha glucosidase inhibitory activities of ethanolic and aqueous extract of whole plant of *Alternanthera Pungens Kunth.* Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia this may be due to defects in insulin secretion, insulin action or both. Alpha amylase and alpha glucosidase inhibitors are used to control hyperglycemia in type 2 diabetes mellitus. The dried whole plant was subjected for extraction with ethanol (95% v/v) by continuous hot soxhletion apparatus and cold maceration with distilled water (0.25 % chloroform). The ethanolic and aqueous extracts of plant were used to study alpha amylase and alpha glucosidase activity, where Acarbose was used as a positive control. A significant effect of ethanolic and aqueous extract in alpha amylase and alpha glucosidase were observed. The alpha amylase inhibition activity of ethanolic and aqueous extracts of Plant showed IC_{50} value of 6.96±1.47 and 7.54±0.73 µg/mL respectively when compared with acarbose (IC_{50} value 7.77±1.42) and in alpha glucosidase assay, the inhibition activity of plant extracts have IC_{50} values 76.78±1.76 and 70.62±1.28_µg/mL respectively when compared to acarbose (IC_{50} value 81.85±1.46). As the extracts contains flavonoids which could be good candidate for inhibition of alpha amylase and alpha glucosidase enzymes. The present study supports the traditional use of this plant in treatment of Diabetes.

***Keywords*:** Diabetes mellitus, *Alternanthera Pungens Kunth,* Alpha-Amylase, Alpha Glucosidase.

Article Info: Received 15 Oct 2018; Review Completed 05 Dec 2018; Accepted 28 Dec 2018; Available online 10 Jan 2019

Cite this article as:

Mourya P, Singh G, Jain N, Gupta MK, In-vitro studies on inhibition of alpha amylase and alpha glucosidase by plant extracts of alternanthera *Pungens kunth*, Journal of Drug Delivery and Therapeutics. 2018; 8(6-A):64-68

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INTRODUCTION

Diabetes mellitus (DN) is a chronic disorder resulting from a variable interaction of hereditary and environmental factors, characterized by damaged β- cells of the pancreas and an increased risk of complication of vascular disease[1]. It is a group of metabolic disease characterized by hyperglycemia, altered metabolism of lipids, carbohydrate and protein this may be due to defects in insulin secretion, insulin action, or both[2]. Type 2 diabetes mellitus is by far the commonest form of the diseases globally, with rarely developing countries being at the forefront as for the epidemic concerned[3]. Type 1 diabetes mellitus with exogenous insulin and type 2 with synthetic oral hypoglycemic agents and /or insulin[4]. Through different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is an increasing demand by patient to use natural products with blood glucose lowering activity[5]. The use of ethanobotonicals has a long folkloric history for the treatment of blood glucose abnormalities.Therefore the researchers continue looking for more effective and safer hypoglycemic agents from natural source.This new era of important area of active research[6].

Alternanthera Pungens Kunth (family Amarathaceae) A spiny prostrate branched weed of roadside, waste land and arid open regions. Leaves of the same pair unequal, obliquely elliptic to orbicular, with flowers and fruits through the year[7]. The whole plant is used in gastric, hepatic and intestinal disturbances. The aerial part is used as diuretics and emollient. Saponins, alkaloid, steroids, triterpenoids, leucoanthocyanidins, β-spinasterol, saponins heteroside of oleanolic acid and choline has been previously described from this plant[8]. The aim of the present study is to investigate the hypoglycemic activity of plant extracts by inhibition of metabolic enzymes.

MATERIALS AND METHODS

Plant collection and preparation

The plant Alternanthera Pungens Kunth was collected from Jabalpur district, Madhya Pradesh, India. This species forms dense mats of stems and leaves during the rainy season. During the dry season or in drought, material above ground dies off and the dormant plant is sustained by its fleshy taproot. The plant was authenticated by the Dept. of Pharmacognosy of Oriental University Indore M.P. Voucher samples were deposited in the herbarium for reference. The

fresh whole Plant was dried under shade and crushed into coarse powder with mechanical grinder. The powder was passed through sieve no.30 and kept in air tight container for further use.

Drug and Chemical

The drugs and chemicals are used for the study are of analytical grade.

Extraction of plant material

The dried coarse powdered material was defatted with petroleum ether (60-80˚c) in a Soxhlet apparatus by continuous hot Soxhletion. The defatted powder material (marc) thus obtained was further extracted with ethanol (95% v/v) with same method and fresh powder was used for aqueous extraction. Distilled water with chloroform (0.25 %) was used for extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator[5, 9].

Phytochemical Screening

Qualitative analysis of extracts of different solvents was carried out to find out the presence of various phytoconstituents[5, 10, 11].

Test for flavonoids content

A portion of the powdered material was heated with 10 ml of ethyl acetate over a steam bath for 3 min. the mixture was filtered and 4 ml of the filtrate was shaken with 1ml of dilute ammonia solution. Development of yellow coloration is an indication of the presence of flavonoids.

Alpha Amylase Inhibition Activity

Alpha-amylase inhibitory activity of ethanolic and aqueous extracts was carried out according to the standard method[12]. In a 96-well plate, reaction mixture containing 50 µL phosphate buffer (100 mM, pH = 6.8), 10 µL α-amylase (2 U/ mL), and 20 µL the plant extracts in concentration range 20–100 µg/mL was pre-incubated at 37 °C for 20 min. Then, the 20 µL of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated at 37 °C for 30 min; 100 µL of the DNS color reagent was then added and boiled for 10 min. The absorbance of the mixture was measured at 540 nm using Multiplate Reader (Multiska thermo scientific, version 1.00.40). Acarbose was used as a standard at concentrations range of 20–100 µg/mL. Without test substance was set up in parallel as control and each experiment was performed in triplicate manner. The results were expressed as percentage inhibition, which was calculated using the formula:

$$\% \text{ inhibition} = \frac{Abs_{540}\,(\text{Control}) - Abs_{540}\,(\text{Extract})}{Abs_{540}\,(\text{Control})} \times 100$$

Where,

Abs_{540} (Control) is the absorbance of control and Abs_{540} (Extract) is the absorbance of test substance

Alpha Glucosidase Inhibitory Activity

Alpha-glucosidase inhibitory activity of ethanol and aqueous extracts was carried out according to the standard method[13]. In a 96-well plate, reaction mixture containing 50 µL phosphate buffer (100 mM, pH = 6. 8), 10 µL alpha-glucosidase (1 U/mL), and 20 µL of both extracts in concentration range 20–100 µg/mL was pre incubated at 37 °C for 15 min. Then, 20 µL P-NPG (5 mM) was added as a substrate and further incubated at 37 °C for 20 min. The reaction was finished by adding 50 µL Na_2CO_3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose as standard at same concentration was included. Without test substance was set up in parallel as a control and each experiment was performed in triplicate. The results were expressed as percentage inhibition, which was calculated as below mentioned equation. Percentage inhibition is calculated as

$$\% \text{ inhibition} = \frac{Abs_{405}\,(\text{Control}) - Abs_{405}\,(\text{Extract})}{Abs_{405}\,(\text{Control})} \times 100$$

Where,

Abs_{405} (Control) is the absorbance of control and Abs_{405} (Extract) is the absorbance of test substance

Statistical analysis

All the measurements were done in triplicate and results are expressed in terms of mean±SD and IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitors.

RESULTS

Phytochemical studies

The extracts of whole plant was subjected for phytochemical screening which reveals the presence of different compounds in plant extract such as alkaloid, glycoside, flavonoid, saponin, carbohydrate, fixed oil and fat and tannins (Table no. 1).The percentage yields of ethanol and aqueous extracts were 8.6 and 6.4 % w/w respectively.

Table 1: Preliminary phytochemical investigation of plant extracts of *Alternanthera Pungens Kunth.*

Sr. no.	Phytochemical constituents	Different types of Extracts		
		Petroleum ether extract	Ethanolic Extract	Aqueous Extract
1.	Alkaloid	–	+	+
2.	Glycoside	+	+	+
3.	Flavonoids	–	+	+
4.	Carbohydrate	+	+	+
5.	Saponins	–	+	+
6.	Triterpens	–	+	+
7.	Phytosterols	–	+	–
8.	Tannins	–	+	+
9.	Fixed oil & fat	+	+	–
10.	Phenolic compound	–	+	+
11.	Gum and mucilage	–	–	+
12.	Protein and amino acid	–	+	+

Where, + ive (positive) = Present. – ive (negative) = Absent

Alpha-amylase and alpha-glucosidase activity

Ethanolic and aqueous extracts of plant showed IC_{50} value of 6.96±1.47 and 7.54±0.73 µg/ml respectively in the alpha amylase inhibition assay and for alpha-glucosidase the Plant showed IC_{50} values of 76.78±1.76 and 70.62±1.28 µg/ml respectively (Table 2 and 3). The present study indicated that Alternanthera Pungens Kunth could be useful in management of postprandial hyperglycemia.

Table 2. α Amylase inhibitory effects of different extracts of *Alternanthera Pungens Kunth.*

Drug	% inhibition at different concentration µg/ml					IC_{50} (µg/ml)
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Aqueous extract	9.34±0.64	26.26±1.83	34.42±0.63	39.38±1.82	42.74±0.44	7.54±0.73
Ethanolic extract	14.32±1.60	32.84±0.64	37.24±2.76	40.32±1.95	48.22±0.56	6.96±1.47
Acarbose	16.62±1.66	34.62±0.46	39.42±0.57	42.67±1.88	50.62±1.82	7.77±1.42

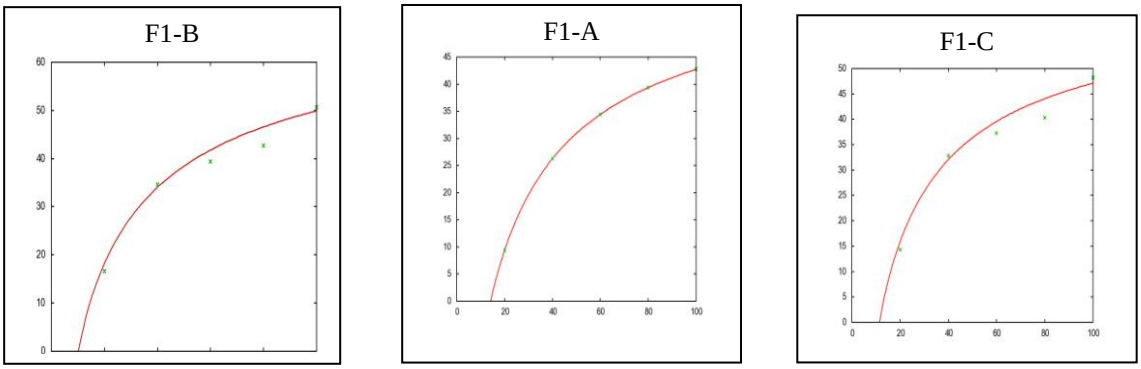

Fig. 1 Alpha amylase inhibition activity. Percent inhibition (Y axis) versus log inhibitor concentration (X Axis).

F1-A: - Alpha amylase inhibition activity by Aqueous extract at different conc.

F1-B: - Alpha amylase inhibition activity by Ethanolic extract at different conc. F1-C: - Alpha amylase inhibition activity by Acarbose at different conc.

Table 3. α Glucosidase inhibitory effects of different extracts of Alternanthera Pungens Kunth.

Drug	% inhibition at different concentration µg/ml					IC_{50} (µg/ml)
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
Aqueous extract	22.46±1.66	32.28±1.44	38.62±0.48	48.44±1.74	51.36±1.63	70.62±1.28
Ethanolic extract	28.64±1.82	36.42±0.76	42.14±0.84	50.34±0.72	54.62±0.83	76.78±1.76
Acarbose	29.44±1.29	39.28±0.65	47.24±1.67	52.32±1.56	57.24±1.23	81.85±1.46

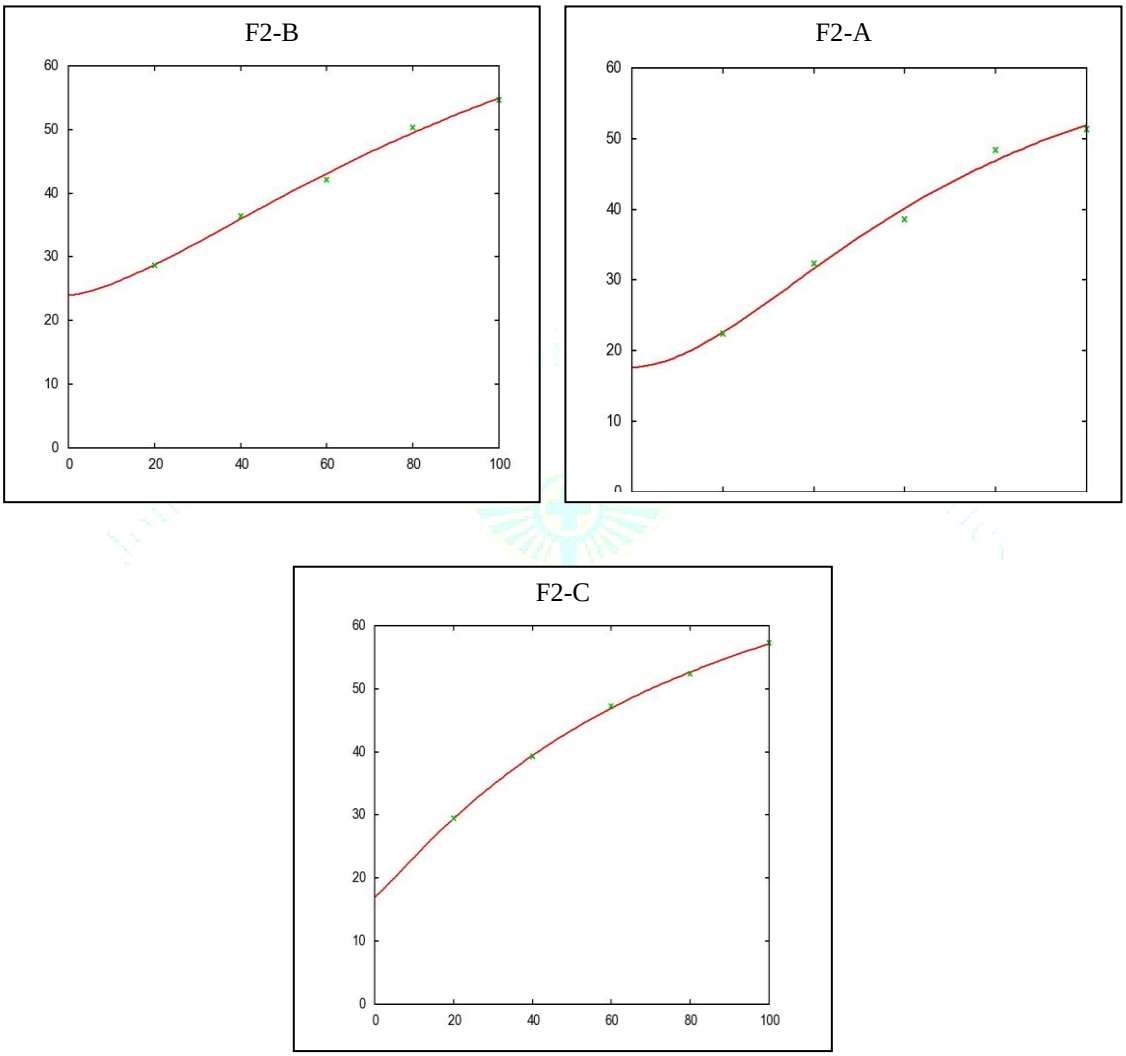

Fig. 2. Alpha Glucosidaes inhibition activity. Percent inhibition (Y axis) versus log inhibitor concentration (X Axis).

F2-A: - Alpha Glucosidaes inhibition activity by Aqueous extract at different conc.

F2-B: - Alpha Glucosidase inhibition activity by Ethanolic extract at different conc.

F2-C: - Alpha Glucosidase inhibition activity by Acarbose at different conc.

DISCUSSION

The extracts of whole plant were subjected for phytochemical screening, which reveals the presence of different compound in plant extract such as Alkaloid, Glycoside and Flavonoid. Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. On the other hand, mammalian α-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. Inhibitors of α amylase and α glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the post prandial blood glucose level[14]. Presently, Acarbose is an important carbohydrate metabolic enzyme inhibitor in the gastrointestinal tract, but it has some side effects such as diarrhea and intestinal disturbances. Therefore, natural plant originated inhibitors have usual much attention. Alpha-amylase is responsible to begin the carbohydrate

digestion process by hydrolysis of 1, 4-glycosidic linkages of polysaccharides to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which support to postprandial hyperglycemia[15,16]. Hence, these enzyme inhibitors are useful to control the hyperglycemia as well as they delay carbohydrate digestion, which reduce the postprandial plasma glucose level. Hence we aimed to evaluate α-amylase and α-glucosidase inhibitory activity of ethanol and aqueous extracts of Alternanthera Pungens Kunth. The results showed that ethanol extract has highest inhibitory potential than aqueous extract.

The presence of flavonoids and phenolics in both extracts might have attributed to the potential enzyme inhibition activity of plant. These results can support the possible mechanism followed by flavonoid compounds to control blood glucose levels by the inhibition of α-amylase and α-glucosidase activity in the intestine[17]. Above results can be correlated with significant enzyme inhibitory activity of ethanol and aqueous extract may interfere or delay the absorption of dietary carbohydrate support to the suppression of diet induced plasma glucose level in the small intestine.

CONCLUSION

The ethanolic extract exhibited the most potent alpha amylase and alpha glucosidase inhibitory activity which is an indication that the solvent is capable of extracting the active constituents from Alternanthera Pungens Kunth. The inhibitory effect of these extracts on both enzymes may be due to presence of flavonoid. This may be beneficial for the development of new antidiabetic agents from native plant resources. Hence, a further investigation was needed to explore the possible mechanism of action.

ACKNOWLEDGEMENT

Authors are very thankful to the Dept. of Pharmacognosy, Oriental University, Indore M.P. for authentication of the plant and also thankful to the administration of University for providing facilities.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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