

Original Research Article

Evaluation of α -Glucosidase Inhibitory Activity of Dichloromethane and Methanol Extracts of *Croton bonplandianum* Baill

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Abstract

Purpose: To evaluate the α -glucosidase inhibitory activity of *Croton bonplandianum* Baill as a probable remedy for the management of diabetes.

Methods: Dichloromethane and methanol extracts were prepared and screened for their α -glucosidase inhibitory activity using standard in vitro α -glucosidase inhibition assay. Acarbose was used as positive control.

Results: α -Glucosidase inhibition activity of dichloromethane extract was 97.89 % with 50 % inhibitory concentration (IC_{50}) of 14.93 mg/ml, while the methanol extract was not active. Acarbose (reference) exhibited 92.23 % inhibition with IC_{50} of 38.25 mg/ml.

Conclusion: The plant contains bioactive compounds with enzyme-inhibiting activity, and thus lends some support for the traditional use of this herb in the management of diabetes. Dichloromethane extract has higher α -glucosidase inhibition activity than the methanol extract. However, further investigation of the plant, including identification of its active components are required.

Keywords: α -Glucosidase, Inhibition, Diabetes, Acarbose, *Croton bonplandianum*

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INTRODUCTION

Diabetes mellitus is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. It is characterized by hyperglycemia in postprandial and fasting state, and its severe form is accompanied by ketosis and protein wasting [1]. Elevated postprandial hyperglycemia (PPHG) is one of the risk factors [2]. PPHG is elevated by the action of α -glucosidase and α -amylase. Inhibition of these enzymes plays a major role in managing PPHG in diabetic patients. Inhibition of α -glucosidase enzyme activity leads to a reduction in

disaccharide hydrolysis which has beneficial effects on glycemic index control in diabetic patients [3,4]. Several α -glucosidase inhibitors have been isolated from medicinal plants for the development of new drugs with increased potency and lower adverse effects than the existing drugs [5].

Croton bonplandianum Baill (syn. *Croton sparsiflorus* Morong) is a monoecious woody shrub, which is 1 - 5 m in height, but more usually c. 30 - 40 cm, with whorled branches. The plant grows in sandy clay soil along roadside, irrigation canal banks, in plantations and on

waste ground [6]. It has been reported that this plant grows in southern Bolivia, Paraguay, southwestern Brazil, northern Argentina, Bangladesh, southern India and Pakistan [7,8]. In Pakistan, this plant is found near Khyber, Attock, Wah, Rawalpindi, Sargodha, Gujarat, Sialkot, Lahore and Karachi.

In previous studies, the present authors found that methanol extracts of *Croton bonplandianum* exhibited antioxidant and cytotoxic activities [9]. However, little work has been done on the enzyme inhibition studies of the plant extract. Therefore, the objective of this study was to assess the α -glucosidase inhibitory activity of the methanol and dichloromethane extracts of *Croton bonplandianum* Baill.

EXPERIMENTAL

Collection and extraction of plant material

The plant material was collected from surroundings of Sargodha district. The plant was identified as *Croton bonplandianum* by Professor Dr Altaf Ahmad Dasti and a specimen voucher (no. SWT-446) was deposited at Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. For the purpose of effective extraction, whole plant material was shade dried for 15 days. Then dried plant material was ground in blender and weighed. The extraction of this finely ground material was affected by simple maceration. The weighed amount of plant material was taken in extraction bottle and measured volume of dichloromethane was added to it. Ultrasonication of this mixture was carried out occasionally in order to achieve maximum possible extraction. Filtration was carried out after 24 h of addition of solvent. The process was repeated three times with dichloromethane. The extraction of the marc was done by methanol in the same manner. The dichloromethane and methanol extracts were concentrated separately under reduced pressure using a rotary evaporator. The dichloromethane (yield, 20.2 g) and methanol (yield, 48.9 g) extracts were collected in separate sample bottles and designated as CBD and CBM, respectively.

In vitro α -glucosidase inhibition assay

The α -glucosidase inhibitory activity was assessed by the standard method [10], with slight modifications. Briefly, a volume of 60 μ l of sample solution and 50 μ l of 0.1 M phosphate buffer (pH 6.8) containing α -glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37

$^{\circ}$ C for 20 min. After pre-incubation, 50 μ l of 5 mM p-nitrophenyl- α -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 $^{\circ}$ C for another 20 min. Then the reaction was stopped by adding 160 μ l of 0.2 M NaCO₃ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 μ l of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The concentrations of test compounds which inhibited the hydrolysis of substrates (butyrylthiocholine) by 50 % (IC₅₀) were determined by monitoring the effect of decreasing concentrations of these extracts in the assays on the inhibition values. The IC₅₀ values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) and mean values were compared by Duncan's Multiple Range Tests using SPSS software, version 15 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

RESULTS

In-vitro α -glucosidase inhibition

The *in vitro* α -glucosidase inhibitory activity of the plant extracts is summarized in Table 1. Dichloromethane extracts possessed α -glucosidase inhibitory activity at 500 μ g/ml concentration. Dichloromethane extract was the most potent, with inhibitory activity of 97.89 % and IC₅₀ of 14.93 mg/ml compared to the standard, acarbose, which exhibited 92.23 % inhibition with IC₅₀ of 38.25 mg/ml.

DISCUSSION

Diabetes is one of the world's greatest health problems, affecting about 171 million people and most of these will be dominated by those suffering from type II diabetes [11]. This increasing trend in type 2 diabetes mellitus has become a serious medical concern worldwide, which accounts for 9 % of deaths that prompts every effort in exploring for new therapeutic agents to stem its progress. Although the drug treatment for type 2 diabetes mellitus has been improved to some extent during the last decade,

Table 1: α -Glucosidase inhibitory activity (mean \pm SEM, n = 3) of methanol and dichloromethane extracts of *Croton bonplandianum*

Extract	Concentration (μ g/ml)	Inhibition (%)	IC ₅₀ (\pm SEM, μ g/ml)
Methanol extract	500	0	-
Dichloromethane extract	500	97.89 \pm 2.6	14.93 \pm 0.37
	250	89.58 \pm 1.6	
	125	79.34 \pm 1.5	
	62.05	68.23 \pm 2.2	
	31.25	60.56 \pm 2.3	
	15.625	51.12 \pm 2.1	
	7.312	40.67 \pm 1.8	
	3.656	30.34 \pm 2.9	
	500	92.23 \pm 0.14	
Acarbose	250	81.39 \pm 0.23	38.25 \pm 0.12
	125	71.09 \pm 0.56	
	62.05	57.42 \pm 0.44	
	31.25	48.02 \pm 0.24	
	15.625	35.99 \pm 0.98	
	7.312	24.87 \pm 1.01	
	3.656	13.09 \pm 1.12	

drug resistance is still a big concern that needs to be dealt with effective approaches. One of the strategies to monitor blood glucose for type II diabetes mellitus is to either inhibit or reduce the production of glucose from the small intestine. Diet rich in carbohydrate causes sharp rise in the blood glucose level as the complex carbohydrates in the food is rapidly absorbed in the intestine aided by the α -glucosidase enzyme which breaks disaccharides into absorbable monosaccharides, α -glucosidase inhibitor inhibits the disaccharide digestion and impedes the postprandial glucose excursion to enable overall smooth glucose profile [12]. Thus, natural products of great structural diversity are still a good source for searching for such inhibitors, thereby motivating to explore biologically active compounds from the highly diverse plants. The pharmaceutical industry is facing serious challenges as the drug discovery process for neurodegenerative diseases is becoming extremely expensive, riskier, and critically inefficient. A significant shift from a single-target to a multi-target drug approach, especially for chronic and complex disease syndromes, is being witnessed. Approaches based on reverse pharmacology (from the clinic to the bedside) also offer efficient development platforms for herbal formulations. The results of the present study indicate that out of the two plant extracts, methanol extracts of *Croton bonplandianum* showed the maximum alpha glucosidase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. The expected bioactive

components could be flavonols or phenolic acids as literature shows a clear link between polyphenols and anti-diabetic activity of herbal extracts [13].

CONCLUSION

In the light of these findings, we can conclude that the plant extracts screened herein showed inhibitory activity against both of the enzymes in dose dependent manner and they could be considered for further studies in the treatment of diabetes and Alzheimer's disease. Further works related to the isolation of the active constituents through bioassay-directed fractionation are in progress in our laboratory.

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