Antiamylolytic activityof okra (Abelmoschus esculentusL.) Pod glycoprotein

Md. Shaharul Islam,Md. Rezaul Karim*,Tan Suat Hian, Shah Samiur Rashid, Md. Shaheen Sarkar

Faculty of Industrial Sciences and TechnologyUniversiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Kuantan, Pahang, Malaysia

*Corresponding author's E-mail: mdrezaul@ump.edu.my

Keywords: Glycoprotein; *Abelmoschus esculentus*; Type 2 diabetes; Antiamylolytic activity; SDS-PAGE.

Abstract

Introduction: The prevalence of diabetes is on a steady increase worldwide and it is now identified as one of the main threats to human health in the 21st century1. There has been an enormous interest in the screening of phytochemicals, specifically for the development of alternative medicines for type 2 diabetes, capable of delaying or preventingstarch hydrolysis and controllingblood glucose level. In Asian countries the okra podis consumed becauseit plays an important role in the human diet by supplying carbohydrates, proteins, vitamins, minerals and asan important source of antidiabetic compounds2. The goal of the present study was to provide in vitro evidence for potential inhibition of α-amylase and α-glucosidase activityby aqueous okra pod extract.

Methodology: Fresh okra pod was cut into small pieces, extracted with double distilled water for 8 h and the extract was precipitated with either 75% ethanol or 5% TCA or 100% ammonium sulphate. The precipitate was collected by centrifugation and used as crude protein extract forthe determination of total protein and carbohydrate content in the extracted sample by the Bradfordmethod3and Resorcinol sulphuric acidassay4,respectively. The protein precipitated by TCA was subjected to SDS-PAGE (Lamlae method) in parallel with a set of MW marker protein to see the molecular size and number of major proteins in the crude extract.

Results& Discussion: The protein contentin different precipitates ranged from 0.428-0.695 µg/g and the carbohydrate content ranged from 0.1-0.128µg/g of freeze driedprecipitate (Table 1). The presence of carbohydrate in the crude protein indicated that the protein is a glycoprotein.The protein precipitated by TCA was dissolved in DMSO/H2O and used for amylolytic inhibition assay. The glycoprotein demonstrated appreciable inhibition against α-amylase and α-glucosidase (Table 2). SDS-PAGE of the protein extract demonstrated 3 distinct bands indicating that the crude extract contained at least 3 proteins of different molecular size (Fig. 1). The approximate molecular weight of the crude proteinsas calculated from the graph (Fig. 2.) were 119, 78 and 60 kDa of which the protein with MW78kDa was the major one. Purification of the glycoproteinsis going on by using Sephadex gel and ion exchange column chromatography.

Table 1: Protein and carbohydrate content in aqueous okra extracts precipitated by ethanol, TCA and (NH4)2SO4

Sample	Protein (µg/g of dry sample)	Carbohydrate (µg/g of dry sample)	Carbohydrate content in protein (%)
Ethanol ppt.	0.428± 0.004	0.128± 0.0007	29.69
TCA ppt.	0.695± 0.003	0.100± 0.003	14.39
$(NH_4)_2SO_4$ ppt.	0.691± 0.02	0.123± 0.001	17.79

All the analyses were done in triplicate and the data are presented as Mean±SD.

Table 2: In vitro α-amylase and α-glucosidase inhibition by aqueous extract fresh okra pod and IC_{50} values

Sample	Enzyme inhibited	Content (µg)	% Inhibition	IC_{50} values (µg)
Okra	α-amylase	40	33.2 ± 0.5	106.89 ± 1.35
		80	42.6 ± 0.2	
		120	52.9 ± 0.1	
		160	65.7 ± 0.3	
		200	72.1 ± 0.6	
	α-glucosidase	40	38.2 ± 0.2	86.80 ± 0.98
		80	47.6 ± 0.3	
		120	58.9 ± 0.1	
		160	68.7 ± 0.4	
		200	80.1 ± 0.3	
Acarbose		10	43.7 ± 0.3	15.14 ± 0.20
		20	55.7 ± 0.2	
		30	68.5 ± 0.1	
		40	80.3 ± 0.2	
		50	92.4 ± 0.1	

All the analyses were done in triplicate and the data are presented as Mean±SD.

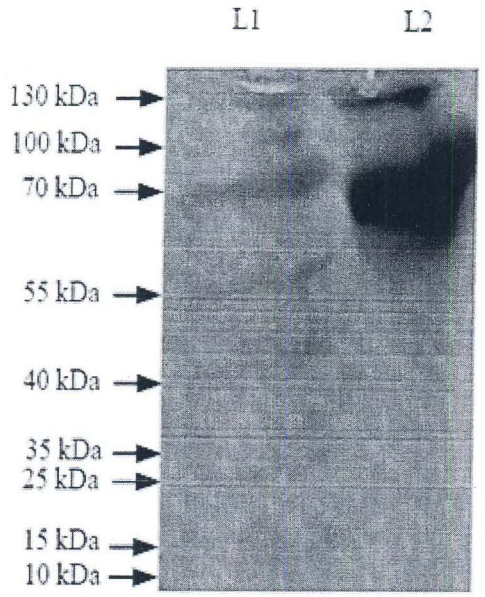

Fig. 1: SDS-PAGE of TCA precipitated protein stained with Coomassie brilliant blue. L1= MW marker protein and L2 = TCA precipitated protein.

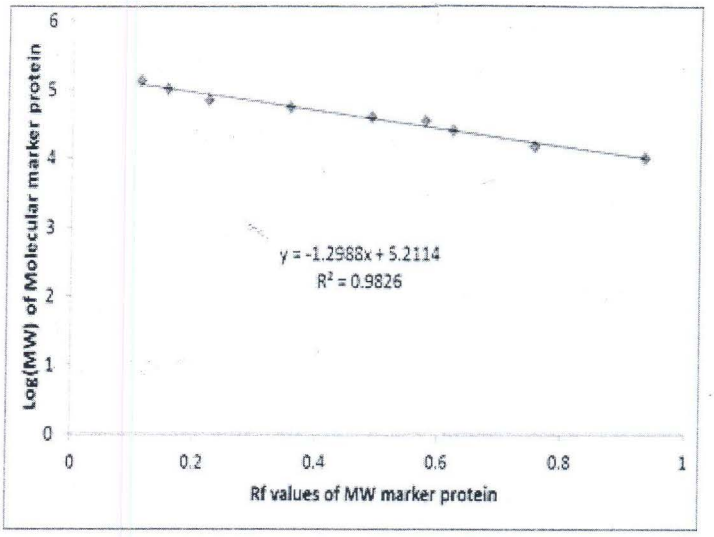

Fig. 2. Standard curve of MW marker protein for MW determination of of the crude proteins.

Acknowledgement: The authors cordially acknowledge that this work was done with the financial support from RDU grant No.110389.

References

1IDF Diabetes Atlas, sixth edition 2014. http://www.idf.org/diabetesatlas/update-2014 (last accessed 02.12.2014).

2Sabitha V., Panneerselvam K. and Ramachandran S. 2012.In vitro α-glucosidase and α-amylase enzyme inhibitory effects in aqueous extracts of Abelmoscus esculentus (L.) Moench, Asian Pacific J Trop Biomed, S162-S164.

3Bradford M. M. 1976."Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Anal Biochem,72, 248–254.

4Monsigny M., Petit C. and Roche A.C.1988.Colorimetric determination of neutral sugars by a resorcinol sulfuric acid micromethod,Anal Biochem,175, 525-530.