Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(6): 201-205
Received: 16-09-2021
Accepted: 18-10-2021

Vijaya Lakshmi T
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Samiksha U
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Nikitha V
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Prashanthi U
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Jyothi V
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Praneetha P
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

Corresponding Author:
Vijaya Lakshmi T
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Osmania University, Hyderabad, Telangana, India

In vitro alpha-amylase and alpha-glucosidase inhibitory activities of methanolic extract of pointed guard (*Trichosanthes dioica*)

Vijaya Lakshmi T, Samiksha U, Nikitha V, Prashanthi U, Jyothi V and Praneetha P

Abstract
Background: Diabetes mellitus is a clinical condition characterized by hyperglycaemia in which an elevated amount of glucose circulates in the blood plasma leading to diabetic compications. Alpha amylase and alpha glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. Pointed guard also known as Parwal (*Trichosanthes dioica*) is a vegetable consumed mostly by the people of all parts of India. In the present study the methanolic extract of *Trichosanthes dioica* was studied for *in-vitro* alpha (α)-amylase and alpha (α)-glucosidase inhibitory activities. **Materials and Methods:** The methanolic extract of fruits of *Trichosanthes dioica* (TDME) was prepared by maceration. In alpha-amylase activity, alpha-amylase solution (0.5 mg/mL) and substrate, 1% starch was used, and absorbance was measured at 540nm. In Alpha-glucosidase activity, alpha-glucosidase (0.5 mg/mL) and substrate, 5 mM p-nitrophenyl-alpha-D-glucopyranoside was used; absorbance was recorded at 405 nm.
Results: Different concentrations of *Trichosanthes dioica* were assessed for alpha amylase and alpha-glucosidase inhibitory activities with an IC_{50} value 8.220mg/ml and 5.819mg/ml extract respectively and were well comparable with the standard drug, acarbose. Conclusion: The extract, TDME, exhibited significant alpha-amylase and alpha-glucosidase inhibitory activities in dose dependent manner and was comparable to that of standard drug, acarbose.

Keywords: alpha amylase, alpha glucosidase, diabetic complications, IC_{50}, *Trichosanthes dioica*

Introduction
Diabetes mellitus is an important chronic metabolic disorder that affects the metabolism of carbohydrate, fat and protein. This disarray in carbohydrate, protein, and fat metabolism lead to micro-and macro-vascular changes causing secondary complications. These secondary complications include heart attack, stroke, kidney failure, leg amputation, vision loss, and nerve damages (Nagamani *et al*., 2013) [1]. Among 7.7 billion total populations (2019), around 463 million adult people have diabetes with a global prevalence of 9.3% and may rise to 10.9% by 2045 (Belma *et al*., 2019) [2]. The three main types of diabetes are type 1, type 2, and gestational diabetes. Both women and men can develop diabetes at any age (Imam, 2015). The only therapy of type 1 diabetes is the substitution of insulin. Many and diverse therapeutic strategies for the treatment of type 2 diabetes are known. The conventional treatments for diabetes include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides (Groop *et al*., 1997) [4]. One group of drugs introduced in the management of type 2 diabetes is represented by the inhibitors of α-glucosidase. The enzymes, α-glucosidase is responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibitory action of this enzyme leads to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine (Bischcoff H, 1994) [5]. Another effective method to control diabetes is to inhibit the activity of α-amylase enzyme which is responsible for the collapse of starch to more simple sugars (dextrin, maltotriose, maltose and glucose) which results in increased glucose levels (Alexander, 1992) [6]. Some inhibitors currently in clinical use are acarbose, miglitol and voglibose etc. However, many of these synthetic hypoglycemic agents have their limitations, are non-specific, produce serious side effects and fail to elevate diabetic complications. The main side effects of these inhibitors are gastrointestinal *viz*., bloating, abdominal discomfort, diarrhea and flatulence. Recently, herbal medicines are gaining more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycemic agents (Grover, 2002) [7].

Trichosanthes dioica is a vine plant of Cucurbitaceae family, perennial and dioecious. The fruits are green with white or no stripes (Shahana *et al*., 2018) [8]. The present study was carried out to investigate the in- *vitro* α-glucosidase and α-amylase inhibitory activities on methanolic extract of fruits of *Trichosanthes dioica*.

Materials and Methods

Chemicals and reagents: Porcine pancreatic α-amylase (EC 3.2.1.1) (PPA) and α –glucosidase, 3,5-Dinitrosalicylic acid (DNSA color reagent), Soluble starch, p-nitro phenyl- α -D-glucopyranoside (p-NPG), were obtained from SRL Laboratories (Hyderabad, India). Acarbose from Glucobay (Hyderabad, India), sodium potassium tartarate, dimethylsulfoxide, sodium carbonate (Na_2 CO_3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate and other chemicals are of analytical grade.

Collection of Plant material: Fresh fruits of *Trichosanthes dioica* (1 kg) were collected from the local area of Hyderabad, Telangana (India). The collected plant material is authenticated by Dr. Mustafa, Professor, Department of Botany, Kakatiya University, Warangal, Telangana, India. The fruits were washed thoroughly under tap water to remove all impurities. Then they were cut into pieces and dried under shade for three weeks. The dried fruits were powdered using grinder.

Preparation of plant extract: The air-dried and coarse powdered sample of fruits of *Trichosanthes dioica* (1 kg) was macerated with methanol in a round bottom flask for 7 days with intermittent stirring and filtered after seven days and concentrated under reduced pressure to yield a dark green semi solid mass. The percentage yield of the extract was found to be 8.4%. The extract is kept in desiccators to remove moisture and used. It is given code as TDME (*Trichosanthes dioica* methanolic extract*)*

Preliminary phytochemical screening of the extract TDME: The methanolic extract of fruits of *Trichosanthes dioica* (TDME) was subjected to various test tube reactions to detect the different classes of phytoconstituents present in it (Kokate *et al*., 1997) [9].

Determination of *in-vitro* alpha-amylase enzyme inhibitory activity of TDME: The inhibition of α -amylase activity was determined according to the method (Kim *et al*., 2011) [10] descried in the literature with minor modifications. Stock solution of extract was prepared by dissolving up to 100mg of each extract in 10ml of dimethyl sulfoxide. A total of 250µl of extracts of varying concentrations(1, 2, 4, 8,10mg/ml) was placed in a tube and 250µl of 0.02M sodium phosphate buffer (pH-6.9) containing α-amylase solution (0.5mg/ml) was added. This solution was pre-incubated at 25°C for 10min. After which 250µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at particular time intervals and then further incubated at 25°C for 10min. This reaction was terminated by adding 500µl dinitro salicylic acid (DNS) reagent. The tubes were kept in boiling water bath for 5min and then cooled to room temperature. The reaction mixture was diluted with 5ml of distilled water and the absorbance was measured at 540nm using UV- Visible spectrophotometer (Shimadzu UV-1800). Acarbose was used as positive control. A control or blank was prepared using the same procedure replacing the extract with distilled water. The concentration of the extract or standard required to inhibit 50% of α -amylase activity under the assay conditions was defined as the IC_{50} value.

The percentage inhibition was calculated using the formula:

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

Determination of alpha-glucosidase enzyme inhibitory activity of TDME: The inhibition of α -glucosidase activity was determined according to the method (Kim *et al*., 2011) [10] described in the literature with minor modifications. One mg of α -glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8). To 100 µl of plant extracts of varying concentrations (1, 2, 4, 8, 10, mg/ml), 200 µl α -glucosidase were added and the mixture was incubated at 37 ºC for 20 min. To the reaction mixture, 100 µl of 3mM p–nitro phenyl-α -D-glucopyranoside (*p*-NPG) was added and incubated at 37 ºC for 10 min. The reaction was terminated by the addition of 2ml of 0.1M Na_2CO_3 solution and the α-glucosidase activity was determined spectrophotometrically at 405 nm using UV-Visible spectrophotometer (Shimadzu UV-1800) by measuring the quantity of p-nitro phenol released from *p*-NPG. Acarbose was used as positive control for α -glucosidase inhibitory activity. A control or blank was prepared using the same procedure replacing the extract with distilled water. The concentration of the extract required to inhibit 50% of α -glucosidase activity under the assay conditions was defined as the IC_{50} value.

The percentage inhibition was calculated using the formula:

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

Results

Preliminary phytochemical screening of the extract TDME: The methanolic extract of fruits of *Trichosanthes dioica* has found to contain various phytoconstituents of medicinal importance like flavonoids, polyphenols, saponins, steroids alkaloids and glycosides etc. Preliminary phytochemical screening of the extract, TDME is presented in Table 1.

***In-vitro* alpha-amylase enzyme inhibitory activity of TDME:** The results of the study are presented in Table 2 and Fig. 1. In the present study, methanolic extract of *Trichosanthes dioica* inhibited the catalysis of alpha amylase at all concentrations (1, 2, 4, 8, 10 mg/ml) and the percentage inhibition was dose dependent. Among all the test doses, TDME has shown remarkable alpha- amylase enzyme inhibition i.e. 59.4% at 10 mg/ml concentration and it was comparable with the standard drug, acarbose (91.2% inhibition at 10 mg/ml concentration).The IC50 value of the extract (TDME) and standard (Acarbose) was found to be 8.22 mg/ml and 3.683mg/ml respectively.

***In-vitro* alpha-glucosidase enzyme inhibitory activity of TDME:** The results of the study are presented in Table 3 and Fig. 2. The extract, TDME was assessed for alpha-glucosidase enzyme inhibitory activity at different concentrations ranging from (1-10 mg/ml) and it exhibited potent α-glucosidase inhibitory activity in a dose dependent manner comparable

with that of the standard drug, Acarbose. The IC_{50} value of the extract (TDME) and standard (Acarbose) was found to be 5.819 mg/ml and 3.640 mg/ml respectively.

Table 1: Phytochemical screening of methanolic extract of fruits of *Trichosanthes dioica*

Classes of Phytoconstituents	TDME
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids/Triterpenoids	+
Carbohydrates	+
Glycosides	+
Tannins	+

(+) indicates presence and (-) indicates absence of the phyto-constituents

Table 2: *In-vitro* alpha-amylase enzyme inhibitory activity of TDME

S. No	Name of sample	Concentration (mg/ml)	% Inhibition	IC_{50} value (mg/ml)
1.	TDME	1	32.3±0.02	8.220
		2	40±0.04	
		4	52.5±0.06	
		8	74.84±0.08	
		10	91.62±0.05	
2.	Acarbose	1	12.8±0.04	3.683
		2	18.43±0.06	
		4	21.07±0.04	
		8	47.18±0.05	
		10	56.09±0.03	

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-amylase inhibitor. TDME: methanolic extract of *Trichosanthes dioica*

Table 3: *In-vitro* alpha-glucosidase enzyme inhibitory activity of TDME

S. No	Name of sample	Concentration (mg/ml)	% Inhibition	IC_{50} value (mg/ml)
1.	TDME	1	45±0.05	5.819
		2	95.08±0.03	
		4	53.85±0.08	
		8	87±0.09	
		10	38.12±0.02	
2.	Acarbose	1	10.2±0.05	3.640
		2	14.99±0.07	
		4	17.34±0.08	
		8	76±0.09	
		10	64.07±0.04	

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-glucosidase inhibitor. TDME: methanolic extract of *Trichosanthes dioica*

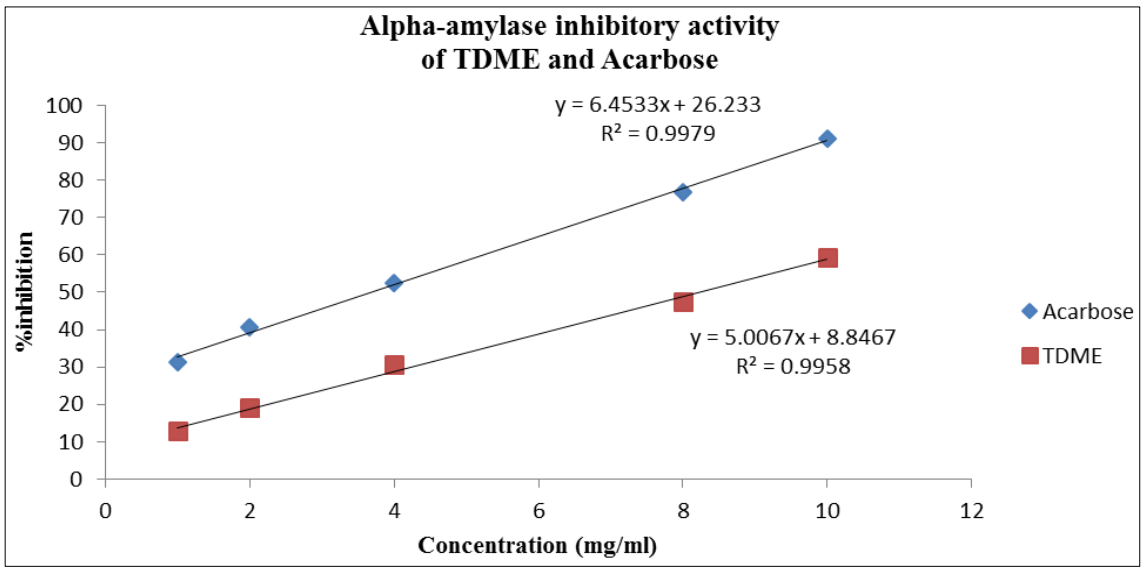

Fig. 1. *In-vitro* α-Amylase inhibitory activity of TDME and Acarbose
TDME: methanolic extract of *Trichosanthes dioica*

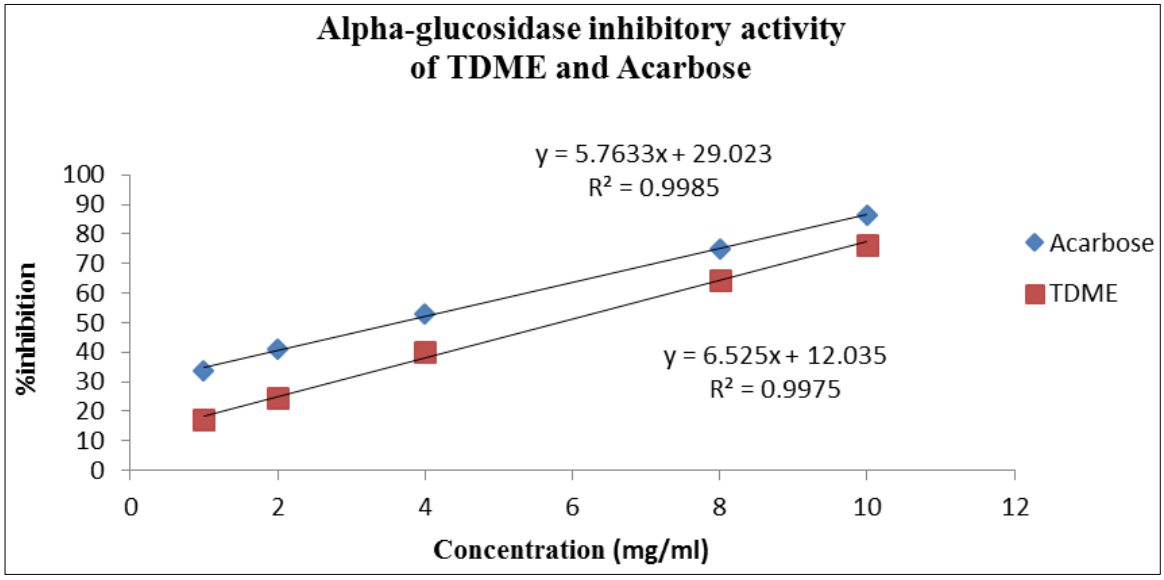

Fig. 2. *In-vitro* α-glucosidase inhibitory activity of TDME and Acarbose
TDME: methanolic extract of *Trichosanthes dioica*

Discussion

The methanolic extract of fruits of *Trichosanthes dioica* has found to contain various phytoactive constituents of medicinal importance like flavonoids, polyphenols, saponins, steroids, alkaloids, and glycosides etc of which flavonoids and polyphenols are responsible for antioxidant activity (Praneetha *et al*., 2008) [11]. The levels of oxidative stress are greater in diabetics resulting in greater requirements of antioxidants to combat oxidative stress (Maxwell *et al*., 1997) [12]. Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides which in turn leading to increase in blood glucose levels. The α-amylase inhibitors, delays the glucose absorption rate thereby decreasing the serum blood glucose levels in hyperglycemic individuals (Dineshkumar *et al*., 2010) [13]. The extract, TDME has shown dose dependent inhibition of alpha amylase enzymatic activity indicating antidiabetic potential of the extract. On the other hand, mammalian -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. α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia (Bischcoff, 1994) [5]. The extract, TDME has shown significant inhibition of alpha glucosidase enzymatic activity indicating anti-hyperglycaemic activity of the extract. α-amylase and α-glucosidase inhibitors play an important role in controlling high blood glucose levels. Inhibitors of alpha -amylase and alpha-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion and hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduce the postprandial plasma glucose level. The extract, TDME has shown dose dependent inhibition of both alpha amylase and alpha glucosidase enzymatic activities indicating antidiabetic potential of the extract.

Conclusion

In the present study, TDME has shown dual inhibition of alpha-amylase and alpha-glucosidase enzymes which in turn decreases blood glucose levels, making the extract effective in the management of diabetes and in turn reduces diabetic complications. The antidiabetic activity may be attributed due to the presence of various phyto-active constituents present in the extract like flavonoids, polyphenolic compounds etc. Hence, the TDME can be used as an adjuvant for the management of diabetes and thus prevent the complications associated with diabetes mellitus.

Acknowledgements

Authors are very thankful to principal and management of Sarojini Naidu Vanita Pharmacy Maha Vidyalaya for providing necessary facilities.

Conflict of interest

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

References

1. Nagamani M, Swaroopa RV. *In vitro* Studies on the Inhibition of alpha-Amylase and alpha-Glucosidase by Root Extract Fraction of *Kyllinga triceps* (rottb). International Journal of Pharmaceutical Sciences and Nanotechnology 2013;6(4):32-36.
2. Belma M, Suvi K, Pouya S, Paraskevi S. International Diabetes Federation: IDF *Diabetes atlas*. 9th ed. Flagship publications, Belgium, 2019, 35.
3. Imam SK. Diabetes- a new horizon and approach to management. In Glucose intake and utilization in pre-diabetes and diabetes. Elsevier Academic press, United Kingdom, 2014, 55.
4. Groop L, Forsblom C, Lehtovirta M. Characterization of the prediabetic state. American Journal of Hypertension 1997;10:172-180.
5. Bischcoff H. Pharmacology of α-glucosidase inhibition. European Journal of Clinical Investigation 1994;24(3):3-10.
6. Alexander R. Maltodextrins, production, properties and applications. In Starch hydrolysis products, worldwide technology: production and applications (Schenk F, Hebeda R eds), New York, 1992, 62-122.
7. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology 2002;81:81-100.

8. Shahana S, Nikalje APG. Effect of *Trichosanthes dioica* aqueous fruit extract in diabetes and diabetic complications. International Journal of Pharmaceutical Sciences and Research 2018;9(6):2540-44.
9. Kokate CK, Purohit AP, Gokhale SB. Analytical pharmacognosy: phytochemical investigations, 5th ed. Niraliprakashan, India.
10. Kim JS, Hyun TK, Kim MJ. The inhibitory effects of ethanol extracts from sorghum, foxtail millet and proso millet on α -glucosidase and α -amylase activities. Food Chemistry 2011;124:1647-51.
11. Praneetha P, Swarooparani V, Narasimhareddy Y, Ravikumar B. Hepatoprotective studies on methanolic extract of whole plant of *Lindernia ciliata.* Bangladesh Journal of Pharmacology 2008;9:567-74.
12. Maxwell SRJ, Thomason H, Sandler D, Lefhien C, Baxterl MA, Thorpe GHG, *et al*. Poor glycaemic control is associated with reduced serum free radical scavenging (antioxidant) activity in non-insulin-dependent diabetes mellitus. Annals of clinicalal Biochemistry 1997;34:638-644.
13. Dineshkumar B, Mitra A, Manjunatha M. A comparative study of alpha amylase inhibitory activities of common antidiabetic plants of Kharagpur 1 block. International Journal of Green Pharmacy 2010;4:115-21.