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Research Article

INHIBITORY EFFECT OF A STANDARDIZED HYDROETHANOLIC EXTRACT OF *TERMINALIA ARJUNA* BARK ON ALPHA-AMYLASE ENZYME

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ABSTRACT

Objective: The present study was initiated to screen the hydroethanolic bark extract for α-amylase inhibitory activity and standardization of the *Terminalia arjuna* for polyphenolic phytochemicals using high-performance liquid chromatography-photo diode array (HPLC-PDA) method.

Methods: The *T. arjuna* bark sample was extracted with ethanol: water (70:30 v/v) using Soxhlet extraction. A Dionex P680 HPLC system was used to acquire chromatograms. The screening of extract of *T. arjuna* bark has performed for *in vitro* α-amylase inhibitory assay. Each experiment was repeated 3 times. All values were expressed mean ± standard deviation.

Results: The content of arjunetin, arjungenin, gallic acid, ellagic acid, and quercetin was 0.47, 8.22, 2.443, 7.901, and 3.20 mg/g, respectively, in a hydroethanolic extract of *T. arjuna*. The hydroethanolic extract of *T. arjuna* bark and acarbose has shown an inhibitory activity with an IC_{50} value 145.90 and 62.35 µg/mL, respectively.

Conclusion: The hydroethanolic extract *T. arjuna* bark demonstrates α-amylase inhibitory activity due to a synergistic effect of the phytochemical constituents present in it. This study suggests that one of the mechanisms of this plant for antidiabetic activity is through the inhibition of α-amylase enzyme.

Keywords: *Terminalia arjuna*, Acarbose, Diabetes, Enzyme inhibition, Arjunetin, Arjungenin.

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INTRODUCTION

Diabetes mellitus (DM) is a complex disease developed due to unbalanced carbohydrate, protein, and fat metabolism. A progressive metabolic disorder finally leads to micro- and macro-vascular changes causing secondary complications [1]. According to the World Health Organization, 346 million people worldwide have diabetes and among 90% of them suffer from type 2 diabetes (T2DM) [2,3]. Type 1 diabetes results from the deficient synthesis of insulin by β-cells of the pancreas, while T2DM is developed due to insulin resistance condition or β-cell dysfunction [1].

The treatment of T2DM is based on oral antidiabetic drugs include sulfonylureas, biguanides, and thiazolidinediones. However, they have demonstrated some side effects which suggest the need for other alternative therapy [4-6]. One of the treatment approaches is to decrease the after-meal blood glucose concentrations [5]. Inhibition of the α-amylase enzyme in the intestine delays the degradation of starch before absorption that reduces postprandial blood glucose concentrations [4,7].

Voglibose, acarbose, and Miglitol are the α-amylase inhibitory drugs available in the market. These are prescribed as combinatorial therapy with sulfonylurea and metformin for the treatment of T2DM to reduce glycated hemoglobin (HbA1c) levels. However, the previous studies have reported few side effects such as abdominal discomfort, flatulence, and diarrhea for these drugs [8]. The side effects caused and drug resistance is two major concerns in the usage of these drugs after prolonged treatment [2].

Traditional medicinal plants have used to treat various diseases. Plant extracts or phytochemical constituents have been reported scientifically for biological activities [5]. *Terminalia arjuna* Roxb. (Combretaceae) commonly known as Arjuna, has been traditionally used for several medicinal purposes such as cardiac diseases, dyslipidemia, and lowering blood glucose [9-11]. The hydroethanolic extracts of the *T. arjuna* (TAHA) leaves and stem bark have been reported to possess anti-diabetic activities in diabetic rats [9,12]. The previous study by Saha *et al*. have reported the α-amylase inhibitory activity of methanolic, aqueous and 50% methanolic extract of *T. arjuna* [13,14]. While there is no evidence in the literature that has determined TAHA bark extract containing arjunetin, arjungenin, ellagic acid, gallic acid, and quercetin compounds for α-amylase inhibition activity. This study will be useful to determine one of the possible mechanisms of effect of TAHA as antidiabetic activity. Therefore, the objective of the present study was to screen the inhibitory activity of TAHA bark extract for α-amylase enzyme and standardization for polyphenolic phytochemicals using high-performance liquid chromatography-photo diode array (HPLC-PDA) method.

MATERIALS AND METHODS

Materials/chemicals

Solvents such as HPLC grade methanol and acetonitrile (Merck, Mumbai, and India) were used to prepare samples and mobile phase, respectively. Water was purified using the Milli-Q (Millipore, Billerica, MA) system. Arjunetin, arjungenin, gallic acid, ellagic acid, and quercetin were purchased as HPLC markers from the Natural Remedies Pvt. Ltd. Bangalore, India. Enzyme α-Amylase was purchased from Sigma-Aldrich, Bangalore, India.

Plant material and extraction

The *T. arjuna* bark sample was collected from a local market in Pune, India, and authenticated by Agharkar Research Institute, Pune, India. The sample was deposited as voucher specimens no. S/B-109. The dried bark was extracted with ethanol: water (70:30 v/v) using Soxhlet

extraction for 8 h for consecutive 3 days at 65°C. The extract was dried under vacuum using rotary evaporator at 45°C and stored at 2–8°C until use.

Estimation of arjunetin and arjungenin in a TAHA bark extract

A Dionex P680 HPLC system including autosampler and thermostatted column compartment and a Dionex UVD 170U/340U photodiode array detector (Dionex Corp., Germering, Germany) was used to acquire chromatograms. The column used was RP C18 BDS Hypersil column (250 × 4.6 mm, 5 µm particle size) from Thermo Electron Corp. (Bellefonte, PA). Chromeleon 6.70 software from Dionex was used to acquire data for fingerprint analysis.

The analysis was performed with an RP C18 BDS Hypersil column (250 × 4.6 mm, 5 µm particle size) at a column temperature 26°C. The gradient program for pump A (acetonitrile: water, 30:70) and pump B (acetonitrile: water, 70:30) as follows: Initially 30% B, flow rate 0.8 ml/min; increased gradually to 50–70% B up to 30 min, flow rate 1.2 ml/min, then 20 min, 30% B, flow rate 0.8 ml/min. The detection wavelength was 220 nm. Injection volume for standard and sample was 20 µl [15]. Data analysis was performed by variations and similarity observed in retention time values, peak areas and spectral patterns of the peaks obtained in the chromatograms of *T. arjuna* bark extract.

Estimation of ellagic acid, gallic acid, and quercetin in a TAHA bark extract

Phytochemical characterization was carried out using gallic acid, ellagic acid, and quercetin by HPLC method [16-18]. Briefly, prominence HPLC system (Shimadzu, Japan) was used. Chromatographic separations were carried out using C-18 column (150 × 4.6 mm, 5 µm particle size; Syncronis, Thermo Scientific, USA). Following gradient elution with water containing 0.5% acetic acid (component A) and acetonitrile: water containing 0.5% of acetic acid (80:20 v/v) as component B was used.

The nonlinear gradient elution program: 0–10 min 10% of B; 10–20 min 20% of B; 20–30 min 40% of B; 30–40 min 60% of B; 40–45 min 70% of B; and 45–55 min 10 % of B and equilibrated with initial conditions for another 5 min. The flow rate and oven temperature were used at 1.0 ml/min and 25°C, respectively. All chromatograms were monitored at 270 nm.

Assay of alpha-amylase inhibition

A mixture of 50 µl of extract or acarbose and 50 µl 0.02 mol sodium phosphate solution (pH 6.9 with 6 mmol sodium chloride) containing alpha-amylase solutions (13 U/ml) were incubated at 25°C for 10 min. After pre-incubation, 50 µl 1% soluble starch solution in 0.02 mol sodium phosphate solution (pH 6.9 with 6 mmol NaCl) was added to each well at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min followed by addition of 1 ml dinitrosalicylic acid color reagent. Then, the test tubes were placed in hot water (at 80°C for 10 min) to stop the reaction. The reaction mixture was diluted with 1 ml distilled water and absorbance was read at 540 nm [19,20].

Statistical analysis

Each experiment was repeated 3 times, and the data averaged for reporting. All values were expressed mean ± standard deviation (SD). Percentage of inhibition was calculated using the formula:

% Inhibition = [(Abs control - Abs sample)/Abs control] *100

The IC_{50} values were determined through nonlinear regression by fitting to a sigmoid dose-response equation with variable slope using GraphPad Prism5 software (GraphPad Software, Inc. La Jolla, CA, USA).

RESULTS

Phytochemical standardization of TAHA bark extract

Phytochemical standardization of TAHA extract was carried out using selected marker-based approach. Arjunetin, arjungenin, gallic acid, ellagic acid, and quercetin were used as phytochemical markers for standardization purpose. The marker contents were estimated using earlier reported HPLC-PDA method suitably modified on column and mobile phase gradient. The optimized chromatographic conditions showed good resolution of the all the peaks. The presence of marker contents in the TAHA extract was identified using retention time and ultraviolet (UV) spectra matching with corresponding reference standards. The retention time for arjunetin and arjungenin was found to be 4.95 and 7.68 min, respectively. The t_R for gallic acid, ellagic acid, and quercetin was found to be 2.36, 24.71, and 31.98 min, respectively. The spectral overlays for arjunetin and arjungenin showed the presence of UV spectra at 220 nm. The spectral overlays for gallic acid, ellagic acid, and quercetin showed the presence of UV spectra at 270 nm. The calibration plots of arjunetin and arjungenin versus peak area were constructed in the range of 31.25–1000 µg/ml. The calibration plots of gallic acid, ellagic acid, and quercetin versus peak area were constructed in the range of 3.125–100 µg/ml. The content of arjunetin and arjungenin was 0.47 and 8.22 mg/g in the TAHA extract (Table 1). These quantitative estimations were consistent with earlier reports on a TAHA extract. The content of gallic acid, ellagic acid, and quercetin was 2.443±0.090, 7.901±0.786, and 3.20±0.351 mg/g, respectively, in the TAHA extract (Table 1). These quantitative estimations were consistent with earlier reports of a TAHA extract.

Effect of TAHA extract and acarbose on α-amylase activity

In this study, acarbose is used as a standard drug for α-amylase inhibitor assay. Acarbose (at a concentrations 5–160 µg/ml) showed α-amylase inhibitory activity from 7.57±1.40 to 76.86±3.72% with an IC_{50} value 62.35±5.39 µg/ml (Table 2).

The percentage inhibition of the TAHA extract on α-amylase enzyme was studied in a concentration range of 10–320 µg/ml. The TAHA extract has shown inhibition of from 8.43±1.51 to 71.00±4.66% with an IC_{50} value 145. 90±16.34 µg/ml. The IC_{50} value for a TAHA extract was 145.90±16.34 µg/ml which is lesser to standard anti-diabetic drug acarbose which was 62.35±5.39 µg/ml (Table 2).

DISCUSSION

Many herbal extracts have reported and used in Ayurveda for the treatment of diabetes. However, medicinal plants have not gained much importance due to lack of proper standardization of herbal medicines and scientific support [5].

Several reports on the phytochemical analysis of *T. arjuna* have been recently published. The major isolated compounds include the five oleane derivatives, namely, arjunic acid, arjunolic acid, arjungenin, arjunetin, and arjunglucoside I from stem bark extract [21]. Also, flavon-3-ols such as (+)-catechin, (+)-gallocatechin, and (-)-epigallocatechin; phenolic acids such as gallic acid, ellagic acid and its derivatives such as 3-O-methyl-ellagic acid 4-O-β-D-xylopyranoside, and 3-O-methyl ellagic acid 3-O-rhamnoside were reported [22]. However, not much data are available related to α-amylase inhibition.

Effective synthetic α-amylase inhibitors (acarbose and voglibose) are available but cause various negative gastrointestinal symptoms

Table 1: Quantitation of arjunetin and arjungenin in the TAHA extract

Compound name	Rt (min)		(mg/g of TAHA)
	Reference standard	TAHA	
Arjunetin	4.95±0.0	5.09±0.02	0.59±0.04
Arjungenin	7.69±0.0	7.88±0.04	0.85±0.01
Gallic acid	2.36±0.0	2.36±0.05	2.44±0.09
Ellagic acid	24.71±0.0	24.71±0.06	7.90±0.79
Quercetin	31.99±0.0	31.97±0.1	3.20±0.35

Results were expressed as mean±SD; n=6, SD: Standard deviation

Table 2: Effect of acarbose and TAHA extract for α-amylase inhibitory activity

Drug/extract	Concentration of acarbose (µg/ml)	% of inhibition	IC_{50} value (µg/ml)
Acarbose	0	0	62.35±5.39
	5	7.57±1.40	
	10	15.43±1.27	
	20	23.71±1.60	
	40	39.00±2.65	
	80	52.86±1.77	
	160	76.86±3.72	
TAHA	0	0	145.90±16.34
	10	8.43±1.51	
	20	13.71±1.60	
	40	20.86±2.34	
	80	33.43±5.06	
	160	50.86±2.67	
	320	71.00±4.66	

Results were expressed as mean±SD; experiments were performed 3 times in triplicate; n=9, SD: Standard deviation

and hepatic disorders [23,24]. Phenolic α-amylase inhibitors from herbal extracts are potentially safer, and therefore, may be a suitable alternative for inhibition of carbohydrate breakdown and control of glycemic index of food products.

While there is no published report in the literature to date that has tested TAHA extract containing arjunetin, arjungenin, ellagic acid, gallic acid, and quercetin compounds for α-amylase inhibition activity. However, the methanolic, aqueous and 50% methanolic extract of *T. arjuna* was reported for α-amylase inhibition activity [13,14]. Table 2 represents the inhibitory effect of different concentration of the TAHA extract and acarbose. A TAHA extract and acarbose exhibited α-amylase inhibitory effects with an IC_{50} value of 145.90±16.34 and 62.35±5.39 µg/ml. In this study, acarbose (10–160 µg/ml) extract has shown an inhibition ranging from 15% to 76% and *T. arjuna* (10–160 µg/ml) though exhibited a minimum inhibitory potential ranging from 8% to 50% against α-amylase, it was less and not statistically significant. These investigations suggest that this may be due to the presence of potential α-amylase inhibitors.

In a recent study, methanol and free flavonoids extract of *T. arjuna* has been reported for α-amylase inhibitory effects with an IC_{50} valve 5.16 and 38.28 mg/ml [13]. Previously, Saha *et al*., reported that methanolic, aqueous and 50% methanolic extract of *T. arjuna* showed α-amylase inhibitory activity. IC_{50} value of methanolic, aqueous and 50% methanolic extract of *T. arjuna* was 1503±0.71 µg/ml, 592±0.34 µg/ml, and 302±0.55 µg/ml [14]. Further, our results suggest that the health promoting benefits of *T. arjuna* herbs used in traditional Indian medicine for treatment of DM may involve the α-amylase inhibiting activity of polyphenolic compounds, and that α-amylase inhibiting activity may be promoted by mechanistic synergies among the present phenolic substances.

Previous studies on the *in vivo* antidiabetic potential of *T. arjuna* bark in Wistar rats concluded that the hydroethanolic extract of this plant possessed strong glucose-lowering property in both alloxan and streptozotocin-induced diabetic rats [12,25], but the mechanism of action remained elusive. The present study suggests that one of the mechanisms by which *T. arjuna* exhibited its hypoglycemic potential in the reported animal study is through the inhibition of α-amylase. This inhibitory activity of the TAHA extract might be due to the presence of several polyphenols such as arjungenin, arjunetin, arjunolic acid, arjunic acid, quercetin, gallic acid, and ellagic acid in it.

The results of this study direct further research to evaluate the therapeutic effect of *T. arjuna* in the management of postprandial hyperglycemia in T2DM either alone or a combinatorial therapy. Further research is needed to investigate the potential α-amylase inhibitory activity of polyphenolic compounds present in *T. arjuna* and at elucidating putative phenolic synergies that may promote inhibition of α-amylase activity.

CONCLUSION

The TAHA bark extract demonstrates good α-amylase inhibitory activity due to a synergistic effect of the phytochemicals present in it. This study suggests that one of the mechanisms by which this plant displayed its antidiabetic potential is by the inhibition of α-amylase.

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AUTHORS CONTRIBUTION

Shushant A Shengule, has majorly performed the experiments in the laboratory. Sanjay Mishra, has provided the experimental concept, data analysis, and sincerely authored the article. Shweta Bodhale, has role for experimental protocol and conducting the experiment along with mentorship.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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