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Research Article

# *In vitro* α- Amylase and α- Glucosidases Inhibitory Effects of Some Plant Extracts

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**ABSTRACT**
Diabetes Mellitus is a metabolic disorder characterized by high blood glucose level caused due to deficiency of insulin secretion or insulin action. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy in the postprandial blood glucose level in patients with type II diabetesPlants contains different chemical constituents with potential for inhibition of α-amylase and hence maybe used as therapeutic. three plants of *Teucrium* species of were tested for α-amylase inhibition. Different concentrations of extracts were incubated with enzyme substrate solution and the activity of enzyme was measured. Also Acarbose was used as the standard inhibitor.Three plant extracts showed inhibition of α-amylase and α-glucosidases, The α-amylase inhibitory activities of extracts were *Phoenix dactylifera* > *Quercus brantii* > *Capparis spinosa*. And also α-amylase inhibitory activities were *Q. brantii* > *Ph. dactylifera* > *C. spinosa.* According to the results, *Ph. dactylifera* inhibited the activity of alpha-amylase with an $IC_{50}$ of 1.73mg/ml. *Q.brantii* with an $IC_{50}$ value of 7.54mg/ml was the second most active of the species tested. *Capparis spinosa* with an $IC_{50}$ value of 12.57mg/ml was less active. *Q. brantii* extract exerted the highest inhibitory activity against alpha-glucosidase, with $IC_{50}$ value of 7.19mg/ml. *Ph. dactylifera* and *C. spinosa* extract exhibited $IC_{50}$ values of 12.21mg/ml and 14.63mg/ml against alpha-glucosidase.

**Keywords:** *Q. brantii*, *Ph. dactylifera*, *C. spinosa* ,*α*-amylase inhibitory effects, Diabetes Mellitus

## INTRODUCTION

Diabetes mellitus is a serious community health problem worldwide. It is several diseases with various etiologies which is identified by derangements in carbohydrate, protein and also fat metabolism due to the completion or relative insufficiency of insulin secretion or insulin action[1,2]. Various kinds of hypoglycaemic elements like biguanides , glycosidase inhibitors and sulphonylurea , aldosereductase inhibitor , thiazolidinediones and carbamoylmethyl benzoic acid are found along with insulin for the therapy of diabetes mellitus[3] Postprandial hyperglycemia is a significant risk factor for acute and chronic complications related to diabetes[4] and so managing postprandial plasma glucose level is crucial in the early treatment of diabetes mellitus and in decreasing chronic complications[5]. An efficient approach for type 2 diabetes control is the inhibition of α-amylase and α-glucosidases. Inhibitors of α-amylase and α-glucosidase delay the breaking down of carbohydrates in the small intestine and reduce the postprandial blood glucose expedition[6,7]. There are many studies of demonstrated or used and designed enzyme inhibitors and also its influence on blood glucose ranges after food uptake[8,9]. Additionally, α-amylase and α-glucosidases inhibitors are one type of the anti-diabetic medicine groups, which Acarbose is the most popular. These types of drugs have a very powerful advantage and are suitable for healing diabetes mellitus[10], but also stimulate gastrointestinal negative effects that reduce their utilize in a Preventive strategy[11]. Appropriately , several researchers are evaluating and developing nutritional an approach to absolutely manage postprandial hyperglycemia, without leading to harmful events in the digestive tract , Medicinal herbs are often considered to be less toxic and free of side effects compared to the synthetic types[12] Screening of α-amylase and α-glucosidase inhibitors from plants and synthetic sources are improving . Inhibitory of these enzymes have been recently discovered from natural scours[13].In this study , three Iranian antidiabetic plants were selected on the basis of their utilize in traditional medications , experimentally decided hypoglycaemic activity in vivo , deficiency of detailed information on hypoglycaemic ingredients and their availability . Extracts of these species were tested for their inhibition of α-amylase and α-glucosidase.

## MATERIALS AND METHODS

*Chemical materials*

**Author for Correspondence*

Table 1. α-Amylase inhibitory activities and IC50 values of different Concentrations

| Plant Extracts | Concentration (mg/mL) | Inhibition (%) | $IC_{50}$ (mg/mL) |
|---|---|---|---|
| | 1.56 | 48.49 ± 0.56 | |
| *P. dactylifera* | 3.12 | 61.17 ± 1.1 | 1.73 |
| | 6.25 | 73.89 ± 1.03 | |
| | 12.5 | 85.57 ± 0.67 | |
| | 25 | 96.26 ± 1.34 | |
| | 1.56 | 24.14 ± 0.23 | |
| *Q. brantii* | 3.12 | 35.52 ± 0.35 | 7.54 |
| | 6.25 | 46.92 ± 1.12 | |
| | 12.5 | 58.30 ± 0.23 | |
| | 25 | 69.68 ± 1.38 | |
| | 1.56 | 32.37 ± 0.77 | |
| *C. spinosa* | 3.12 | 38.33 ± 1.12 | 12.57 |
| | 6.25 | 44.31 ± 1.78 | |
| | 12.5 | 49.67 ± 0.81 | |
| | 25 | 56.23 ± 0.22 | |

Table 2. α-glucosidase inhibitory activities and IC50 values of different Concentrations

| Plant species Extracts | Concentration (mg/mL) | Inhibition (%) | $IC_{50}$ (mg/mL) |
|---|---|---|---|
| | 1.56 | 35.95 ± 0.23 | |
| *P. dactylifera* | 3.12 | 40.71 ± 1.08 | 12.21 |
| | 6.25 | 45.49 ± 0.73 | |
| | 12.5 | 50.25 ± 0.37 | |
| | 25 | 55.02 ± 1.11 | |
| | 1.56 | 29.14 ± 0.08 | |
| *Q. brantii* | 3.12 | 41.07 ± 1.02 | 7.19 |
| | 6.25 | 53.04 ± 1.11 | |
| | 12.5 | 64.97 ± 0.57 | |
| | 25 | 76.91 ± 1.45 | |
| | 1.56 | 31.99 ± 0.64 | |
| *C. spinosa* | 3.12 | 37.46 ± 0.33 | 14.63 |
| | 6.25 | 42.94 ± 0.34 | |
| | 12.5 | 48.41 ± 1.03 | |
| | 25 | 53.88 ± 1.23 | |

All chemical substances were obtained from Sigma Aldrich (USA) and also Merck (Germany) companies. The chemicals were of analytical grade.

*Plant materials*

Young whole plants were collected from Ahvaz Province, Iran in May 2007. The plant was botanically identified and authenticated by local Plant Biotechnologist, Department of Natural Resources, khuzestan, Iran. The plants were dried at ambient temperature (30–40°c) for 25–30 days Then plants were into fine powder.

*Extraction and fractionation procedure*

The dried and powdered plants (100 g) were extracted with ethanol 90% v/v through soxhlet apparatus. The crude extracts were filtered and concentrated under reduced pressure at approximately 40°C.

*Examination of α-amylase inhibition*

The α-amylase inhibitory activity assay was performed using altered methods of Oboh et al . 2013 , Suitable dilutions of the vegetable extracts ( 500 µl ) and 500 µl of 0 .02 mol/L sodium phosphate buffer ( pH 6 .9 with 0 .006 mol/L NaCl ) including pancreatic α-amylase ( EC 3 .2 .1 .1 ) ( 0 .5 mg/ml ) were incubated at 25°C for 20 min . After that , 250 µl of 1% starch solution in 0 .02 mol/L sodium phosphate buffer ( pH 6 .9 with 0 .006 mol/L NaCl ) was included in the reacting mixture . Thereafter , the reaction mixture was incubated at 25°C for 10 min and stopped with 1 .0 ml of dinitrosalicylic acid ( DNSA ) . The mixture was then incubated in a boiling water bath for 5 min and cooled to room temperature . The reaction mixture was then diluted by adding 10 ml of distilled water , and absorbance calculated at 540 nm in the UV-Visible spectrophotometer ( Japan ).

*Examination of α-Glucosidase inhibition.*

The α-glucosidase inhibitory activity assay was carried out using modified methods of Apostolidis et al. 2007, with some modifications. Appropriate dilution of the plant extracts (100 µl) and 200 µl of α-glucosidase solution was incubated at 25°C for 10 min. Thereafter, 50 µl of 5 mmol/L *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/l phosphate buffer (pH 6.9) was added. The reacting mixture was then in-cubated at 25°C for 5 min before reading the absorbance at 405 nm in the UV-Visible spectrophotometer (Japan).

% Inhibition = [(AbsRef – AbsSamples) / AbsRef] × 100

In this studyAcarbose solution (at the concentrations of 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 µg/ml) was used as positive control. The inhibition percentage of α-amylase and α-glucosidase were assessed by the following formula:

Iα-amylase % =100×(ΔAControl – ΔASample)/ΔAControl

ΔAControl=ATest - ABlank

ΔASample= ATest – Ablank

The inhibitory percent α-amylase and α-glucosidase was plotted against sample concentration and a logarithmic regression curve was obtained in order to calculate the IC50 value which is concentration of sample (mg/ml) necessary to decrease the absorbance of α-amylase and α-glucosidase solution by 50%.

*Statistical analysis*

All values were expressed mean ± SD. Statistical difference and linear regression analysis were performed using SPSS 16 statistical software.

**RESULTS AND DISCUSSION**

Diabetes mellitus is a chronic metabolic disorder identified by hyperglycemia due to insulin insufficiency and/or insulin resistance contributing to excess blood glucose. It affected approximately 171 million people all around the world in the year 2000 and the number is projected to increase to around 366 million in 2030[14]. Diabetic individuals are at an increased risk of developing acute and chronic complications such asretinopathy, nephropathy,and neuropathy[15].Management of the blood glucose level is an essential approach in the control of diabetes complications. α-Amylase and α-glucosidase are key enzymes involved in carbohydrates breakdown and intestinal absorption, respectively. Inhibition of these enzymes hinders blood glucose level increase after a carbohydrate diet and can be an important strategy in the management of non-insulin-dependent diabetes mellitus[16]. Commonly used synthetic inhibitory drugs such as acarbose and miglitol possessed negative effects. Traditionally, various herbs were used directly as a medication. Clinically effective substances are now being obtained from plants, even those that have not been categorized before as medicinal herb. Currently, medicinal herbs due to simple accessibility and also lesser side effects have a unique place in medicine to treat several diseases . In fact , several classes of α-amylase and α-glucosidase inhibitory substances derived from the herb have been observed , for examples hydrolysable tannins , flavonoids , xanthones , fatty acids , terpenoids , peptides , procyanidins ,and caffeoylquinic acid derivatives[17-19].

The *Ph . dactylifera* , *C . spinosa* and *Q . brantii* are traditional medicinal herbs typical in world , specially iran .these plants are widely used in the treatment of hypertension , hyperlipidemia , hyperglycemia, cerebral congestion, ulcers and gastrointestinal diseases ,spleen and liver diseases, Although there are citations of antihyperglycemic and antidiabetic activity of *Ph.dactylifera*, *Q. brantii* and *C. spinosa*[20,21], there are no earlier studies , at the least to our training , on the activity of the genus on in vitro α-amylase and α-glucosidase activity . In present study, the inhibition activities of the extracts obtained from *Ph.dactylifera*, *Q. brantii* and *C. spinosa* were investigated on the α-amylase and α-glucosidase enzymes and IC50 values were calculated. Among the plants studied, three plants, demonstrated inhibitory concentration dependent effects on the α-amylase activity. The high inhibitory effect on α-amylase activity ( at Concentration: 25mg/ml) was shown by the extract of *P. dactylifera* (96.26% with an $IC_{50}$ of 1.73mg/ml) *Q. brantii* and *C. spinosa* extracts revealed a weaker activity, 69.68% and 56.23%, respectively . At Concentration 25mg/ml, *Q.brantii* extract showed the high inhibitory activity against α-glucosidase (76.91% with $IC_{50}$ value of 7.19mg/ml) and also *Ph.dactylifera* and *C. spinosa* exposed a lesser activity, 55.02% and 53.88% , respectively. It is possibly because of the fact that at high extract concentrations, there is certainly a confirmation alter derived from authentic of substances to the enzyme[8,22].The percentage inhibition and IC50 values displayed by each extract is shown in Table 1and 2.The IC50 value of the positive control, acarbose, was measured as 0.04 mg/mL.

All plants studied demonstrated inhibitory concentration dependent effects on the α-amylase and α-glucosidase activity.It may be due to the presence of more chemical constituents such as terpenes , tripenes , flavonoids, alkaloids and etc. in these plant extracts. The plant-based α-amylase and α-glucosidase inhibitor provides a potential therapeutic strategy for the control of hyperglycemia[20,21]. The in vitro α-amylase and α-glucosidase inhibitory effect of these plant extracts, especially *Ph.dactylifera* and *Q. brantii* , suggested it to be a good source of α-amylase and α-glucosidase respectively that could be used in functional food or for antihyperglycemia medication.

Previous research studies on α-amylase and α-glucosidase inhibitors identified from medicinal herbs recommend that a number of capability inhibitors belong to terpenes , tripenes , flavonoids that has features of inhibiting α-amylase and α-glucosidase activities[6]. In general , the enzyme inhibitory activity of plant extracts not just rely on the amount of especial phytochemicals but additionally may depend on the quality of especial phytochemicals . Additional researchers also have reported that biological activities of phytochemicals depend on the extent of hydroxylation and also conjugation[23,24].

In conclusion, among all the herbal extracts tested in this study, *Ph.dactylifera* and *Q. brantii* can be the promising sources of α-amylase and α-glucosidase inhibitors, respectively . However, *C. spinosa* that exhibited moderate activity can be stand on second level of interest.

Further , in vitro and in vivo research are required to confirm the present observations , findings on the isolation of active substances contained in the extract and in vivo studies are necessary to recognize a potential chemical substance entity for clinical utilize in the therapy of diabetes and other related disorders.

*Authors' Contributions*

All authors had equal role in design, work, statistical analysis and manuscript writing.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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