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Research Article

INHIBITORY ACTIVITIES OF *Phoenix dactylifera*, *Capparis spinosa*, *Quercus brantii*, AND *Falcaria vulgaris* HYDROALCOHOLIC EXTRACTS ON A –AMYLASE AND A– GLUCOSIDASE

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Abstract

Diabetes mellitus is a chronic metabolic disorder with a prevalence of 5% in the general community. One therapeutic approach for diabetes treatment is decreasing postprandial glucose. Alpha-glucosidase and alpha-amylase Inhibitors normally use for controlling post prandial hyperglycemia in type 2 diabetic patients. Although acarbose and viglibose currently are used as α-amylase and α-glucosidase inhibitors in the treatment of diabetes mellitus, but their side effects such as flatulence and diarrhea cause limitations for usage of these drugs. The aim of present study was to evaluate the α-glucosidase and alpha-amylase inhibitory potentials of *Phoenix dactylifera*, *Capparis spinosa*, *Quercus brantii* and *Falcaria vulgaris*. The most inhibitory effect on α-glucosidase was detected by *Q. branti* with IC_{50}=7.19 mg/ml. This plant also showed a significant effect on α-amylase inhibition with IC_{50}=7.54 mg/ml. The most inhibitory effect on α-amylase was by *P.dactylifera* pollen grains with IC_{50}=1.7 mg/ml. It showed relatively high inhibitory effect on α-glucosidase with IC_{50}=12.2mg/ml as well.

Keywords: *Phoenix dactylifera*, *Capparis spinosa*, *Quercus brantii*, *Falcaria vulgaris*, Alpha-glucosidase Inhibitors, alpha-amylase Inhibitors.

Introduction

Diabetes mellitus is a chronic metabolic disorder with a prevalence of 4-4.5% in Iran. It is greater than 14% in population aged above 30 years. It is estimated that the number of people suffering from diabetes in the world will reach to 300 million up to 2025(Lotfi, Saadati et al. 2013). More than 8.69% of total health expenditure consume for diabetes in additiondiabetes imposes high intangible costs on society by reduced quality of life, thus control of diabetes is a public health priority(Javanbakht, Baradaran et al. 2011).(Lotfi, Saadati et al. 2013)Initial treatment for diabetesis dietary nutrition and physical exercises. If these lifestyle changes were unsucssesful, various anti-diabetic medicine might be used. Many pharmacological approaches are used to improve diabetes via different mechanism of action such as stimulation of insulin release, inhibition of gluconeogenesis, increase the number of glucose transporters and reduction intestinal absorption of glucose(Nikavar, Abou et al.).

One therapeutic approach for diabetes treatment is decreasing postprandial glucose. And the best therapeutic approach to decrease postprandial hyperglycemia is to postpone the absorbtion of glucose in gastrointestinal tract by inhibition of key enzymes

linked to type 2 diabetes (α-amylase and α-glucosidase)(Nikavar, Abou et al.)'(Oboh, Ademosun et al. 2013).

Pancreatic α-amylase is a key enzyme that catalyses first step in starch hydrolyzing to a mixture of small oligosaccharides then these are degraded by α-glucosidase into glucose which is absorbed to the blood stream. Degradation of dietary starch proceeds rapidly and leads to elevated postprandial glucose(Kumar, Kumar et al. 2012).Alpha-glucosidase Inhibitors normally use for controlling post prandial hyperglycemia in type 2 diabetic patients but also they could be useful in the treatment of type 1 diabetes. Although this application isnot approved by FDA,α-glucosidase inhibitors has therapeutic potential for treatment of HIV infection, metastatic cancer and lysosomal storage disease(Gholam Hosseinian, Falah Hossein et al. 2008).Although acarbose and viglibose currently areused as α-amylase and α-glucosidase inhibitors in the treatment of diabetes mellitus(Kumar, Kumar et al. 2012), but their side effects such as flatulence and diarrhea cause limitations for usage of these drugs(Chiasson, Josse et al. 2002).As previos studies are shown plants are good sources for these inhibitors such as *Ascophyllum nodosum, Focus vesiculosus (Kim, Rioux et al. 2014), Anthocleista djalonesis, anthocleista vogelii (Olubomehin, Abo et al. 2013) and Moringa stenopetala (Toma, Makonnen et al. 2014),*

Moreover in previous studies on herbal medicine we have examples such as *Salacia reticulata* which contains kotalanol that has more inhibitory effect on α-amylase than acarbose (Yoshikawa, Murakami et al. 1998, Jayakanthan, Mohan et al. 2009).Thusapart from currently available therapeutic options many herbal medicines have been recommended for this purpose (Kumar, Kumar et al. 2012).

Diabetes treatment by *Quercus* species has been reported in the traditional medicine of some countries such as *Q.coccifera* in Jordan, *Q.alba*and *Q.rubra* in America and Canada, *Q.acutifolia* in mecsico and *Q. lanata* in India(Ahmed, Smithard et al. 1991). In a study *Q. infectoria* showed inhibitory effect on α-glucosidase in one concentration that examined(Gholam Hosseinian, Falah Hossein et al. 2008).Along with this study we demonstrate the inhibitory effect of hydroalchoholic extract of *Q. brantii*on α-glucosidase and α-amylase in several concentrations.

In a study on 400 individuals to investigate the most beneficial traditional plants for treatment of diabetes and hypertension in Morocco, *Phoenix dactylifera* was introduced as one of the 36 beneficial plants in the diabetes treatment(Tahraoui, El-Hilaly et al. 2007).Date fruit was used in traditional medicine of Middle East and Northern Africa for treatment of diabetes(Vayalil 2012),Besides hydroalcoholic leaf extract of *P.dactylifera* on rats showed decreasing effect on serum glucose(Mard, Jalalvand et al. 2010).

Anti-diabetic effect has been reported from Capparidaceae family, Furthermore *Capparis spinosa* and *Cappari siberica* are traditionally used in Morocco as anAnti-diabetic agent(Jarald, Joshi et al. 2008).*C.spinosa* has shown antihyperglycemic effect in mice(Lemhadri, Eddouks et al. 2007).

Falcaria vulgaris is a member of Apiaceae family. Some plants of this family have been shown antidiabetic activities(Jarald, Joshi et al. 2008). This effect could be due to the inhibition of carbohydrate hydrolyzing enzymes. The aim of present study was to evaluate the α-glucosidase and α-amylase inhibitory potentials of *Phoenix dactylifera*, *Capparis spinosa*, *Quercus brantii* and *Falcaria vulgaris*.

Materials and Methods

Plant material

F.vulgaris (Apiaceae) aerial part, *Q.brantii*(Fagaceae) fruits, *P.dactylifera* (Arecaceae) pollen grains and *C.spinosa* (Capparidaceae) fruits were collected from natural fields in Khuzestan and Lorestan provinces of Iran. The plants were identified at the Herbarium of Department of Pharmacognosy, school of pharmacy, Ahvaz, Iran where voucher specimens were preserved. The plants were dried in the shade outdoors in 25-26°C then they were ground. The dried plant materialswere ground and stored in brown glass bottles until extraction at 25°C.

Extraction

The air-dried and ground sample (100g) of each plant powder was extracted with 80% ethanolic solution by maceration at room temperature for 72 hour. The extracts were filtered and concentrated by rotary evaporator. The remained solvent evaporated by oven in temperature below 40°C.

Chemicals

Potato starch, sodium chloride, α-amylase from porcine pancreas (EC 3.2.1.1), α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), maltose, sodium acetate, sodium potassium tartrate, 3,5-dinitrosalicylic acid, o-dianisidine color reagent (DIAN), glucose oxidase peroxidase enzyme solution (PGO), sodium hydroxide, perchloricacid, acetic acid, NaH_2PO_4, Na_2HPO_4, DMSO and acarbose from Sigma-Aldrich Chemie GmbH (Germany).

Alpha-glucosidase inhibition assay

Alpha-glucosidase inhibition assay adopted from Sigma-Aldrich bioassay method by loizzoetal. The plant extracts were dissolved in DMSO to give following concentrations 1.56, 3.125, 6.25, 12.5, 25 mg/ml. One hundred µl of each extract were mixed with 1ml maltose solution 4% and the reaction was started by adding 200µl α-glucosidase solution (2 unit/ml). The tubes were incubated at 37°C and after 30 min 200µl percloric acid solution 4.2% w/v added to stop the reaction. Eighty µl DIAN and 1.2 ml PGO solution added to the 40 µl supernatant solution. They were incubated at 37°C for 30 min. The absorbance at 500nm determined by a spectrophotometer. The negative control was 100µl DMSO and the positive control had following concentrations of acarbose 0.0094, 0.0184, 0.036, 0.07, 0.11, 0.21 µl/ml instead of extract. Alpha glucosidase inhibition percentage was calculated by following equation:

Alpha-amylase inhibition assay

We used modified α-amylase inhibition assay earlier reported by Nickavar et al(Nickavar, Abolhasani et al. 2010). Followingextract concentrations were prepared 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 mg/ml by serial dilution method. The control blank had no extract and no enzyme. Blanks contained 1ml buffer instead of enzyme solution but other contents were such as samples.

One-half ml of sample (extract, positive control (acarbose), negative control (DMSO)) and 0.5 ml of enzyme solution (0.5 unit/ml) were mixed in a tube and incubated at 25°C for 30 min.The mentioned concentration of acarbose was prepared. One ml starch solution 0.5% w/v was added to the tubes. They were incubated at 25°C and after 3min, 1ml color reagent was added. The test tubes were placed in a water bath at 85°C for 15 min. After cooling to room temperature 9ml distilled water was added to the tubes.The absorbance at 540nm was determined by a spectrophotometer.

The inhibition percentage was calculated as follows:

$I_{\alpha\text{-amylase}}\% = 100(\Delta A_{Control} \_ \Delta A_{Sample})/\Delta A_{Control}$

$\Delta A_{Control} = A_{Control} \_ A_{Blank}$

$\Delta A_{Sample} = A_{Test} \_ A_{Blank}$

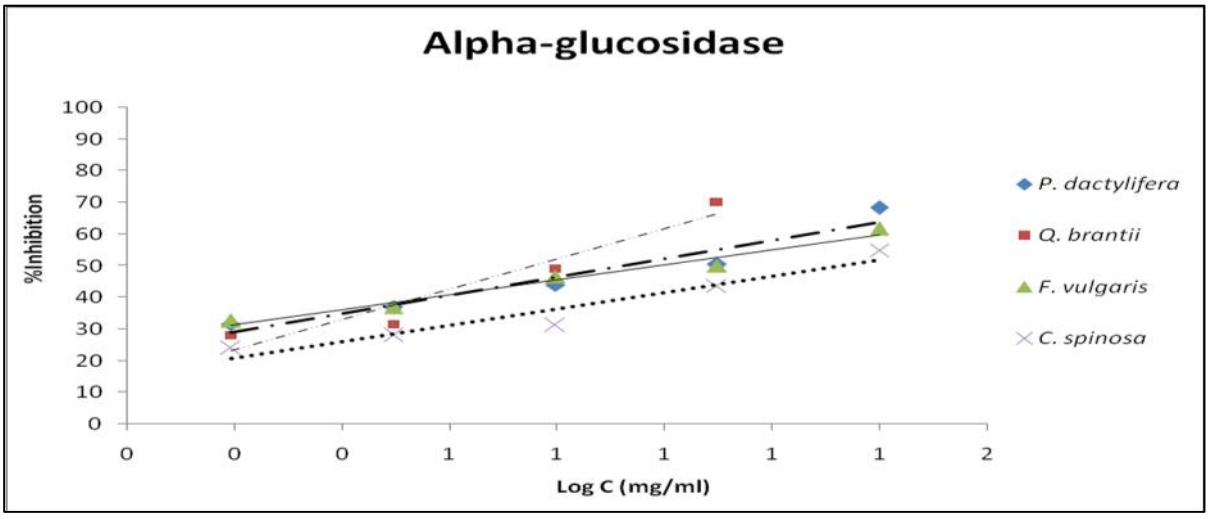
Statistical analysis

All experiments were carried out in triplicates.The curve of percentage inhibition versus concentration was plotted and the linear regression curve established in order to calculate the IC_{50} value. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the *α*- glucosidase and α-amylase inhibitory activity.

Results

We investigated the inhibitory effect of four plant species;*Phoenix dactylifera*, *Capparisspinosa*, *Quercusbrantii* and *Falcaria vulgaris* which showed wide spectrum inhibition between 2.05% and 97.4% on α-glucosidase and α-Amylase. Figure 1 and 2show the inhibitory effect of different concentration of studied plants.

Figure 1. Alpha-glucosidase inhibition percentage of different concentration of *Q.brantii,P.dactylifera*, *F.vulgarid* and *C.spinisa*.



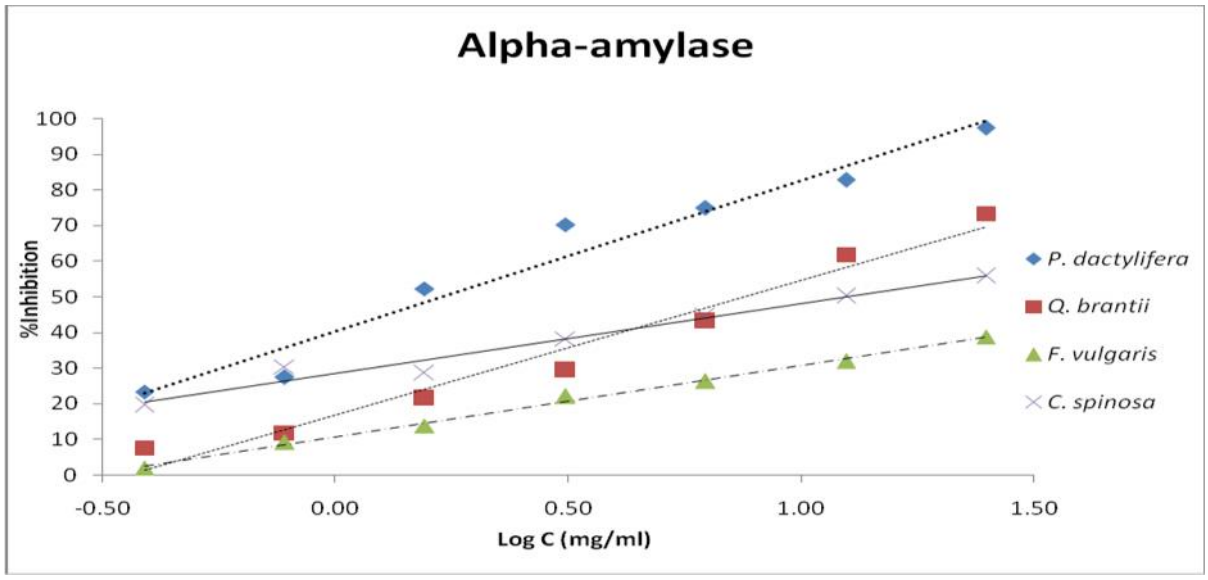


Figure 2. Alpha-amylase inhibition percentage of different concentration of *P.dactylifera,Q.brantii,F.vulgaris, C.spinosa*.

The IC_{50} values were calculated fromα-glucosidase and α-Amylaseinhibition curves obtained with increasing amounts of inhibitor (Figure 3).

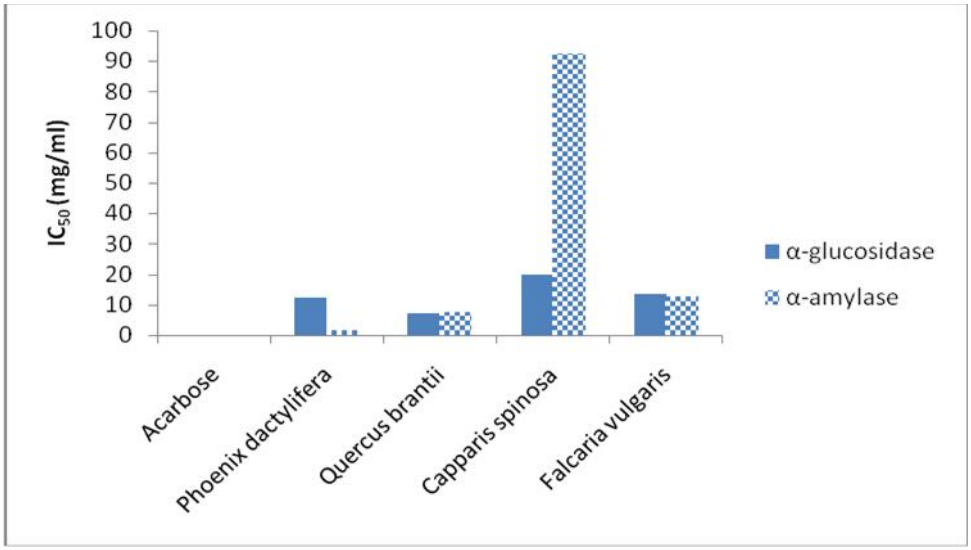


Figure 3.Determination of IC_{50} values of different plants andacarboseasa positive control.

Discussion

One of the therapeutic approaches for diabetes treatment is inhibition of converting carbohydrate food content to glucose in order to reduce post-prandial hyperglycemia. Such kind of treatments usingtheinhibition of key enzymes for carbohydrate hydrolyzing like α-glucosidase and α-amylase. Natural products are good sources of these inhibitors(Kumar, Kumar et al. 2012). The most inhibitory effect on α-glucosidase was detected by *Q.branti* with IC_{50}=7.19 mg/ml. This plant also showed a significant effect on α-amylase inhibition with IC_{50}=7.54 mg/ml. In a study the inhibitory effect of*Q.infectoria* on α-glucosidase in comparison with acarbose as a positive control was investigated.

They revealed that the inhibitory effect of water and methanolic extracts was96±4% and 98±2% respectively, while acarbose showed 51% inhibitory effect further more *Q.infectoria* Grouped in high potency α-glucosidase and α-amylase inhibitors among 200 studied species (Gholam Hosseinian, Falah Hossein et al. 2008).Besides they studied the effect of *Q.infectoria* water extract on post-prandial glucose in either diabeteic and healthy rats, which demonstrated significant decreasing effect on post-prandial glucose in diabeticrats($p<0.0001$) despite healthy rats ($P>0.05$)(Gholam Hosseinian and Falah Hossein).Hexagalloyl glucose extracted from gall methanolic extract of *Q. infectori* a compared with acarbose, showed more inhibitory effects on α-glucosidase and less on α-amylase. Strong inhibitory activity on α-amylase cause more side effects such as diarrhea and abdominal discomfort thus low inhibitory effect on α-amylase by hexagalloyl glucose is beneficial for decrease side effects. In the present study we used different concentrations of hydroalchoholic extract of *Q. branti* on α-glucosidase and α-amylase. Our results authenticated the inhibitory effect of *Q.brantii* and are in agreement with pervious studies(Hwang, Kong et al. 2000). The most inhibitory effect on α-amylase was by *P.dactylifera* pollen grainswithIC_{50}=1.7 mg/ml. It showed relatively high inhibitory effect on α-glucosidase with IC_{50}=12.2mg/ml as well .Date sugar showed inhibitory effect on α-glucosidase (75% in 50µl), α-amylase (100% in 500µl) and angiotensin-converting-enzyme (56% in 50µl) that are due to the high total phenolic and antioxidant activity of the plant (Ranilla, Kwon et al. 2008). Additionally palm pollen grains showed modelatory effect on sex hormones, proteins, lipids and liver functions(El-Desoky, Ragab et al. 1995). Present study suggests that *Q.branti* and *P. dactylifera*exert their anti-diabetic effect by inhibition of α-glucosidase and α-amylase hydrolyzing enzymes.

Capparis spinosa inhibited α-glucosidase and α-amylase with IC_{50}=20.03 and 92.57mg/ml respectively. *C.spinosa* fruit is traditionally used as an anti-hyperglycemic food and it showed anti-hyperglycemic and anti-hypertriglycemic activity in type 2 diabetic patients(Fallah Huseini, Hasani-Rnjbar et al. 2013). Its aquese extract showed anti-hyperglycemic and weight reducing effect in high fat diet obese mice(Lemhadri, Eddouks et al. 2007) and hypolipidemic activity in normal and diabetic rats(Eddouks, Lemhadri et al. 2005).*C. spinosa* hydroalchoholic fruit extract lead to decrease in blood sugar and also a considerable decrease in blood triglycerides in diabetic rats(Rahmani, Mahmoodi et al. 2013).Another study reported antihyperglycemic effect of C.spinosa but non significant decrease in plasma cholesterol and triglyceride(Mishra, Panda et al. 2012). This plant also has been shown antioxidant (Tesoriere, Butera et al. 2007) antihypertensive (Ali, Ali et al. 2007)and anti-inflammatory effects(Al-Said, Abdelsattar et al. 1988).These effects could be useful in diabetes prevention and treatment.

Falcaria vulgaris inhibited α-glucosidase with IC_{50}=13.53mg/ml and α-amylase with IC50=12.57mg/ml. In previous studies *F.vulgaris* showed inhibitory activity on aldose reductase (AR), the key enzyme of the polyol pathway, which plays an important role in the etiology of the complications of diabetes (Enomoto, Okada et al. 2004).Its hydroalchoholic extract was shown an increase in the healing of the peptic ulcer caused by consumed aspirin.(Khazaei and Salehi 2007)Falcarindiolthat is a compound isolated from *F.vulgaris* exhibited significant anti-Candida, antibacterial, and anti-mycobacterial activity.

Conclusion

Acarbose and viglibose currently areused as α-amylase and α-glucosidase inhibitors in the treatment of diabetes mellitus, but their side effects such as flatulence and diarrhea cause limitations for usage of these drugs. Previous investigations on herbal medicine showed good potentional for natural α-amylase and α-glucosidase inhibitors such as kotalanol that isolated from *Salacia reticulata* which has more inhibitory effect on α-amylase than acarbose. Thus apart from currently available therapeutic options many herbal medicines have been recommended for this purpose.

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