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α-Glucosidase and α-amylase inhibitory effect and antioxidant activity of ten plant extracts traditionally used in Iran for diabetes

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In the present study various extracts of ten medicinal plants, collected in Iran, were examined for α-glucosidase and α-amylase inhibition using an *in vitro* model. Also total phenol content and antioxidant activity of the extracts were investigated. Various extracts of the plants (*Cinnamomum zeylanicum, Crataegus oxyacantha, Hibiscus sabdariffa, Morus alba, Portulaca oleracea, Rubus fruticosus, Syzygium aromaticum, Teucrium polium, Trigonella foenum-graecum,* and *Vaccinium arctostaphylos*) were prepared using *n*-hexane, dichloromethane, chloroform, ethyl acetate and methanol. Methanol, dichloromethane and *n*-hexane extracts of *S. aromaticum* exerted high *in vitro* inhibitory potential against α-glucosidase and α-amylase with IC_{50} ranging from 0.3 to 1.1 and 36.2 to 41.9 µg/ml, respectively. The mentioned extracts possessed the highest total phenolic contents (139.8, 119.6 and 136.1 mg GAE/g of extract). The antioxidant activities of the extracts, measured in terms of IC_{50} values were 2.2, 3.9 and 0.7 µg/ml, respectively. *C. zeylanicum* was another traditionally used medicinal plant, which its extracts exhibited high hypoglycaemic effect by inhibition of α-glucosidase and α-amylase (IC_{50} ranged from 0.5 to 8.7 and 37.1 to 52.5 µg/ml, respectively). The obtained results support the traditionally use of a number of the analyzed species.

Key words: α-Glucosidase, α-amylase, enzyme inhibition, diabetes, antioxidant activity.

INTRODUCTION

The incidence of diabetes and obesity is increasing worldwide at an alarming rate, due to changes in modern lifestyles (Tarling et al., 2008; Zimmer et al., 2001). The International Diabetes Federation estimates that 285 million people live with diabetes around the world in 2010. With an increase of about seven million people each year, this total is expected to rise to 438 million within 20 years (www.idf.org). The prevalence of diabetes among Iranians is estimated at approximately 5% (~4 million people) and is increasing at a rate of 1% annually (Iranian Diabetes society, www.ir-diabetes-society.com).

Two types of diabetes mellitus are currently known, one being insulin-dependent diabetes (type 1) and the other being non-insulin-dependent diabetes (type 2). Type 1 diabetes is treated by regular injections of insulin. More than 90% of diabetic population has type 2 diabetes and finding an effective treatment for this type is not easy, due to its non-insulin-dependent nature (Apostolidis and Lee, 2010). It has been demonstrated that maintenance of healthy blood glucose levels is very important for treating type 2 diabetes (Tsujita et al., 2008). The first-line treatment for type 2 diabetes is diet, weight control and physical activity. If blood glucose level remains high despite a trial of these lifestyle measures, then drugs are usually advised. There are several categories of drugs for type 2 diabetes, including sulfonylureas, biguanides, thiazolidinediones, meglitinides, dipeptidyl peptidase IV

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inhibitors and α-glucosidase inhibitors; each works differently (Rang et al., 2003). Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. Pancreatic α-amylase and intestinal α-glucosidase are key enzymes in the digestive system and catalyze the first step in the digestion of starch, hydrolyzing the α-1,4-glucoside linkages. The inhibition of these enzymes significantly decreases the digestion and uptake of carbohydrates, thereby decreasing the postprandial blood glucose level in the non-insulin-dependent diabetes mellitus patients (Fred-Jaiyesimi et al., 2009). Therapeutic drugs, such as acarbose, miglitol and voglibose in current medical are used as α-glucosidase and α-amylase inhibitors. The main drawback of these drugs is their side effects such as abdominal distention, bloating, meteorism, flatulence and possibly diarrhoea (Chakrabarti and Rajagopalan, 2002; Kimmel and Inzucchi, 2005).

It has been suggested that these side effects might be caused by the excessive inhibition of the pancreatic α-amylase, leading to the abnormal bacterial fermentation of undigested carbohydrates in the colon (Horii et al., 1987; Bischoff, 1994). Natural products which have been shown to possess a low inhibitory effect against α-amylase and high inhibition activity against α-glucosidase can be used as an effective means to reduce postprandial hyperglycaemia with minimal adverse effects (Tarling et al., 2008; Kim et al., 2009). Therefore, the search for more effective and safer hypoglycaemic compounds from medicinal plants has continued to be an important area of active research. Several studies have reported the ability of various medicinal plants in inhibition of α-glucosidase and α-amylase (Mayur et al., 2010; Shai et al., 2010; Zhang et al., 2010; Kim et al., 2009; Loizzo et al., 2008; Ali et al., 2006).

It has been demonstrated that about 5% of the inhaled oxygen is converted to reactive oxygen species (ROS), which consist of free radicals such as superoxide (O_2^-), hydroxyl (OH) nitric oxide (NO) and lipid peroxyl (LOO^-) and non-free radical species like hydrogen peroxide (H_2O_2), ozone (O_3) and lipid peroxide (LOOH) (Maxwell, 1995). This ROS produce oxidative stress and generate many pathophysiological disorders such as arthritis, inflammation cancer and diabetes (Mandal et al., 2009). Oxidative stress is due to an imbalance between the free-radical-generation and radical-scavenging capacities (Maritim et al., 2003; Yao et al., 2010). Antioxidants can act as free radical scavengers by preventing and repairing damages caused by ROS and therefore can enhance the immune defense and reduce the risk of degenerative diseases (Pham-Huy et al., 2008).

It has been demonstrated that diabetic patients are under oxidative stress. The increased free-radical generation and reduced antioxidant defense may partially mediate the beginning and progression of diabetes-associated complications (Jin et al., 2008). Therefore,

use of antioxidants can be beneficial for diabetic patients, not only to maintain antioxidants levels in the body but also to treat the long term complications that can arise (Iwai, 2008).

Iran located on the Middle East, presents a climatic and ecological diversity that is unique to the region, and there are a widespread diversity of medicinal plants through the country. The Iranian people can buy herbals in stores called "Attari" where the herbalist suggests plants for specific disease without a prescription. In this study, 10 medicinal plants recommended in "Attari" and traditional Iranian medicine for diabetes, are selected. Various extracts of these species were tasted for their inhibition of α-glucosidase, α-amylase and antioxidant activity. The species used are represented in Table 1.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained from Sigma-Aldrich (Paris, France): α-Glucosidase type I from Baker Yeast (EC 3.2.1.20), porcine pancreas α-amylase (EC 3.2.1.1, type VI), p-nitrophenyl-α-D-glucopyranose (PNPG), 3,5-dinitrosalicylic acid (DNS), maltose, and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Soluble starch, sodium potassium tartarate, sodium dihydrogen phosphate (NaH_2PO_4), sodium chloride, sodium hydroxide, butylated hydroxytoluene (BHT), potassium persulfate, sodium carbonate, Folin-Ciocalteu reagent and gallic acid were purchased from Merck.

Plant material

Cinnamomum zeylanicum (Lauraceae), *Crataegus oxyacantha* (Rosaceae), *Hibiscus sabdariffa* (Malvaceae), *Portulaca oleracea* (Portulaceae), *Syzygium aromaticum* (Myrtaceae), *Teucrium polium* (Lamiaceae), *Trigonella foenum-graecum* (Leguminosae) and *Vaccinium arctostaphylos* (Ericaceae), were purchased from a local market (Attari) in Mashhad, Iran. *Morus alba.* (Moraceae) and *Rubus fruticosus* (Rosaceae) were collected from Mazandaran province in July 2010. The plants were botanically identified by Dr. F. Najafi of Agriculture Department of Medicinal Plants and Drug Research Institute, Shahid Beheshti University, Tehran, Iran. Table 1 show the plant species and family, their traditional uses, part used and local name.

Preparation of extracts

The air-dried and ground samples (30 g) were extracted with 300 ml n-hexane, dichloromethane, chloroform, ethyl acetate and methanol, respectively by maceration on an orbital shaker at room temperature. Solvent was replenished every 24 h for 5 days to ensure that all possible compounds could be extracted. The resulting extracts were filtered and concentrated using a rotary evaporator, under reduced pressure at approximately 40°C. The filtered plant extracts were combined and stored at -20°C until the α-glucosidase and α-amylase inhibition assays. Percent yields (w/w) ranged from 0.79 to 6.20 for n-hexane extracts, from 0.48 to 7.27 for dichloromethane extracts, from 0.07 to 0.83 for chloroform extracts, from 0.22 to 8.02 for ethyl acetate extracts, and from 2.92 to 36.23 for methanol extracts. Significant amount of chloroform

Table 1. Plant species studied.

Plant species	Family	Traditional use	Part used	Local name
C. zeylanicum	Lauraceae	Antibacterial, antioxidant, blood pressure reduction[a], blood sugar reduction [b]	Bark	Darchin
C. oxyacantha	Rosaceae	blood pressure reduction, blood sugar reduction	Leaves	Sorkhevalik
H. sabdariffa	Malvaceae	Antioxidant, blood pressure reduction[d], blood sugar reduction [c]	Flowers	Chay-e-makki
M. alba	Moraceae	Laxative, blood lipid reduction, blood sugar reduction [e]	Leaves	Toot
.P. oleracea	Portulaceae	Antibacterial[f], antidiabetic and antiviral[f], enhancing immunity[f]	Seed	Khorfeh
R. fruticosus	Rosaceae	Antioxidant[g], antirheumatism, blood sugar reduction [h], sedative	Leaves	Tameshk
S. aromaticum	Myrtaceae	Antimeteorism, digestive, sedative	Flowers	Mikhak
T. polium	Lamiaceae	Anticolic, antiseptic, blood sugar reduction [i]	Aerial parts	Kalpoureh
T. foenum graecum	Leguminosae	Antidiabetic[j], antiinflammatory[k], antimelanogenic[k]	Seed	Shanbalileh
V. arctostaphylos	Ericaceae	Blood pressure reduction, blood sugar reduction[l], sedative	Fruits	Qareqat

[a], Wansi et al. (2007); [b], Zari and Al-logmani (2009); [c], Zargari (1993); [d], Wahabi et al. (2010); [e], Oku et al. (2006); [f], Gao et al. (2010); [g] Benvenuti et al.,(2004); [h], Hassimotto et al.(2008); [i], Esmaeili and Yazdanparast (2004); [j] Yadav et al. (2010); [k], Kawabata et al. (2010); [l], Feshani et al. (2010).

extract was not obtained for Darchin (*C. zeylanicum*), Mikhak (*S. aromaticum*) and Qareqat (*V. arctostaphylos*) and ethyl acetate extract for Mikhak and Shanbalileh (*T. foenum graecum*).

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity of the extracts was assessed according to the chromogenic method reported by Ranilla et al. (2010) with slight modifications. The mixture contained 20 µl α-glucosidase (0.5 unit/ml), 120 µl of 0.1 M phosphate buffer (pH 6.9) and 10 µl of test sample at various concentrations. The mixed solution was incubated in 96-well plates at 37°C for 15 min. After pre-incubation, the enzymatic reaction was initiated by adding 20 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) and the reaction mixture was incubated for another 15 min at 37°C. The reaction was terminated by adding 80 µl of 0.2 M sodium carbonate solution and then absorbance reading was recorded at 405 nm by microplate reader (BioTek XS2). The reaction system without plant extracts was used as control and the system without α-glucosidase was used as blank for correcting the background absorbance. The inhibitory rate of sample on α-glucosidase was calculated by the following formula:

$$\%inhibition = \left(\frac{control\ absorbance - sample\ absorbance}{control\ absorbance}\right) \times 100$$

α-Amylase inhibition assay

α-Amylase inhibition activity was determined by measuring the

reducing power of released oligosaccharide from soluble starch by the method of Miller (1959) with some modifications. The assay system which was carried out in 96-well plates, comprised the following components in a total volume of 250 µl: 100 mM sodium phosphate, pH 6.8, 17 mM NaCl, 1.5 mg soluble starch, 50 µl of inhibitor solution in DMSO at various concentrations, and 10 µl of enzyme solution (25 unit/ml). After incubation at 37°C for 30 min, the reaction was stopped by the addition of 20 µl NaOH (2 N) and 20 µl color reagent (4.4 µM of 3,5-dinitrosalicylic acid, 106 µM of potassium sodium tartarate tetrahydrate and 40 µM of NaOH) followed by a 20 min incubation at 100°C water bath. α-Amylase activity was determined by measuring the absorbance of the mixture at 540 nm, using BioTek XS2 microplate reader. Individual blanks were prepared for correcting the blank ground absorbance, where the enzyme was replaced with buffer as follows:

$$Corrected\ Absorbance\ of\ test = Absorbance\ of\ test\ sample - Absorbance\ of\ blank$$

A standard calibration curve was prepared for the maltose in the range of 0 to 0.25% (w/v). The percentage (w/v) of maltose in the reaction wells was calculated from the corrected absorbance of each test and using the equation of the calibration curve. Control incubations, representing 100% enzyme activity were conducted in the same manner replacing the plant extract with DMSO. All experiments were carried out in triplicate and acarbose was used as a reference standard. The percentage of α-amylase inhibition was calculated by the following equations:

$$\%reaction = \frac{mean\ of\ maltose\ in\ sample}{mean\ of\ maltose\ in\ control} \times 100$$

$$\%inhibition = 100 - \%reaction$$

Determination of total phenolic content

The total phenolic content of the extracts were determined using Folin-Ciocalteu assay with minor modification (Zhou et al., 2004). In brief, the reaction mixture contained 2.5 µl of plants extracts, 195 µl of distilled water, 2.5 µl of the Folin-Ciocalteu reagent and 50 µl of sodium carbonate (7%). After 30 min of reaction in the darkness at room temperature, the absorbance was measured at 765 nm with a spectrophotometric microplate reader (BioTek XS2 model). Different concentrations of gallic acid solution were used to establish a standard curve. Results were expressed as miligram of gallic acid equivalents per gram of extract (mg GAE/g of extract). All experiments were carried out in triplicates.

DPPH radical scavenging assay

The DPPH radical scavenging of plant extracts were evaluated as previously described (Xu et al., 2010). 50 µl of various concentrations of test sample including BHT as a reference compound in DMSO were added to 200 µl of 100 µM DPPH solution in methanol with final concentrations of 0.312, 0.625, 1.25, 2.50, 5.00, 10.00, 20.00, 30.00, and 40.00, µg/ml. After an incubation period of 30 min at room temperature in the darkness, the decrease in the absorbance was measured on the microplate reader at 517 nm. The control contained 50 µl of DMSO instead of test sample, and the blank contained methanol in place of DPPH solution. Experiments were carried out in triplicates. The percentage inhibition was calculated by the following equation:

$$\%inhibition = \left[1 - \frac{(Abs^s - Abs^b)}{Abs^c} \right] \times 100$$

where Abs^s is the absorbance of test sample, Abs^b is the absorbance of the blank and Abs^c is the absorbance of control.

Statistical analysis

All assays were performed at least in triplicate and the results were expressed as mean±standard deviation (SD). Statistical analyses were done using SPSS version 18.0. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Tukey's multicomparison test. Differences were considered significant at $p<0.05$. Pearson correlations were used to evaluate the correlation between the various parameters. IC_{50} values were determined by plotting a percent inhibition versus concentration curve for all assays.

RESULTS AND DISCUSSION

α-Glucosidase and α-amylase inhibition activity

In this study, the inhibitory effects of 45 extracts from ten Iranian medicinal plants against α-glucosidase and α-amylase, two well-known key enzymes related to type 2 diabetes were investigated. Figure 1a to e show the IC_{50} values of the extracts with different polarities, against the

enzymes. The IC_{50} values for α-glucosidase and α-amylase inhibitory activities of plants methanolic extracts ranged from 0.3 to 93.2 and 36.2 to 97.5 µg/ml, respectively. Mikhak methanolic extract exerted the highest inhibitory effect against both α-glucosidase and α-amylase enzymes, with IC_{50} of 0.3±0.3 and 36.2±1.2 µg/ml. Broadhurst et al. (2000) found that Mikhak and Darchin aqueous extracts have insulin-like effect by increasing glucose uptake into adipocytes. Prasad et al. (2005) demonstrated that the mentioned effect of Mikhak extract is due to decreasing phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphate (G 6 Pase) gene expression. Among the other analyzed methanol extracts, Darchin, Chay-e- makki, Tameshk (*R. fruticous*), Sorkhevalik, Qareqat and Toot (*M. alba*) extracts exhibited strong inhibitory activity against α-glucosidase with IC_{50} values of 0.5±0.2, 0.7±0.4, 1.1±0.6, 1.5±0.5, 3.9±0.9 and 8.9±1.0 µg/ml, respectively. All mentioned extracts showed stronger inhibition activity in comparison with the reference drug, acarbose (IC_{50}=10.4±0.7 µg/ml). Kalpoureh and Shanbalileh methanol extracts exhibited moderate α-glucosidase inhibitory properties (with IC_{50} values of 14.4±1.6 and 34.1±2.9 µg/ml, respectively) and methanol extract of Khorfeh exhibited low inhibitory activity with IC_{50} at 93.2±0.8 µg/ml. Regarding to α-amylase inhibitory effect of methanol extracts, Mikhak and Darchin extracts possessed relatively high inhibitory effect with IC_{50} of 36.2±1.2 and 37.1±1.0 µg/ml, respectively, as compared with acarbose as control compound (IC_{50}=34.5±2.0 µg/ml). Chay-e-makki, Tameshk and Qaraqat methanol extracts exhibited moderate α-amylase inhibition potency with IC_{50} of 50.5±2.0, 53.7±2.0 and 57.0±0.5 µg/ml, respectively. According to the calculated Pearson correlation coefficients, α-glucosidase and α-amylase inhibition potential of methanol extracts were strongly proportional to the total phenolic contents (r=0.855, $p<0.01$ and r=0.874, $p<0.01$, respectively).

There are several reports about Darchin hypoglycaemic effect. Shen et al. (2010) reported that the aqueous extract of Darchin had antihyperglycemia effect on streptozotocin-induced diabetic rats. They demonstrated that Darchin aqueous extract exhibited its anti-diabetic effect independently from insulin by at least two mechanisms, upregulation of mitochondrial UCP-1 and enhanced translocation of GLUT4 in the muscle and adipose tissues. It has been revealed that both A- and B-type procyanidin oligomers existed in Darchin related species *Cinnamomum cassia* had hypoglycaemic activities and may improve insulin sensitivity in type 2 diabetes mellitus (Lu et al., 2011).
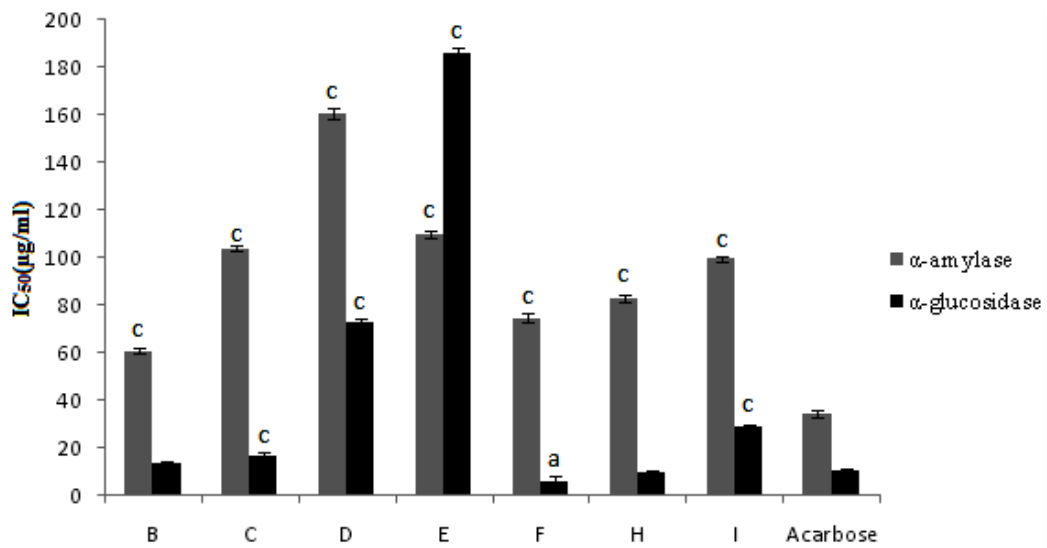
Among analyzed ethyl acetate extracts, Darchin and Sorkhevalik exerted the highest α-glucosidase inhibition activity (IC_{50} at 3.1±0.6 and 7.4±0.7 µg/ml, respectively). Both extracts also inhibited α-amylase activity with IC_{50} of 40.1±0.9 and 68.9±1.2 µg/ml, respectively. α-Glucosidase
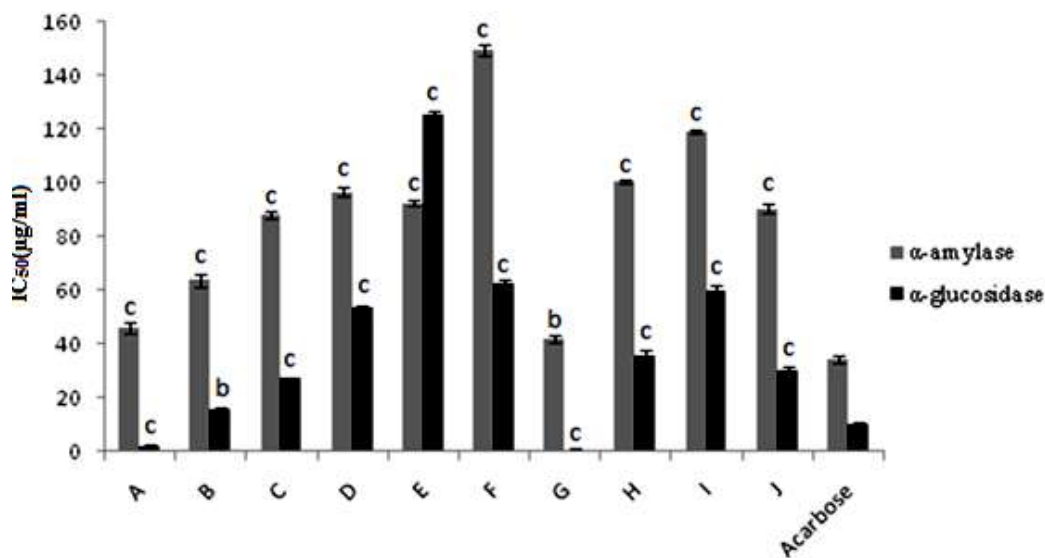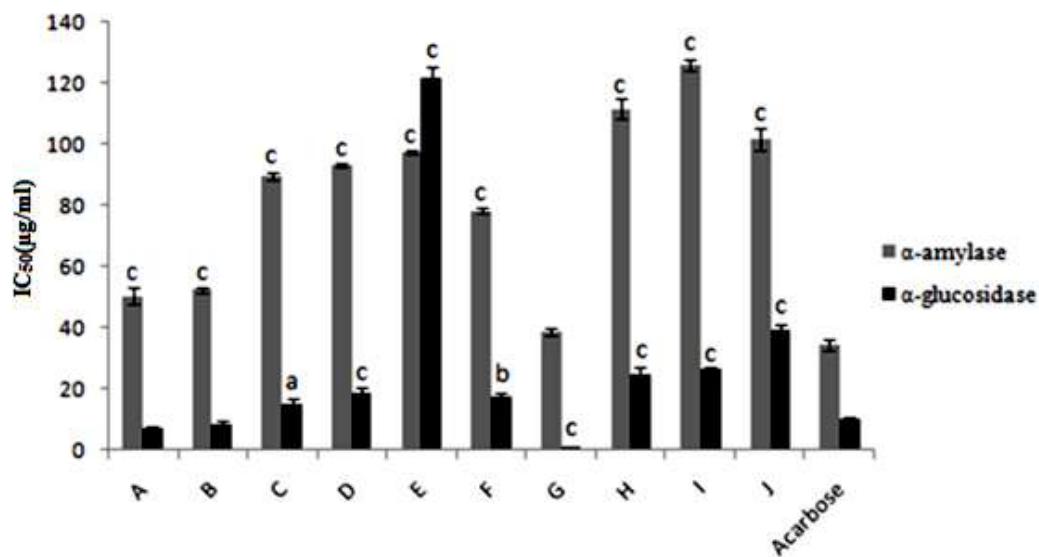
(a)

(b)

(c)

(d)

(e)
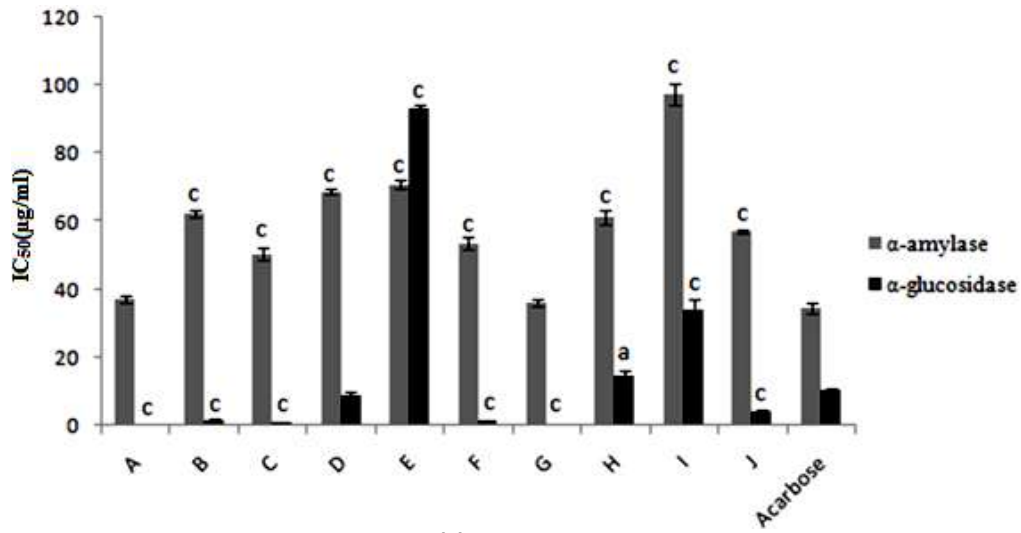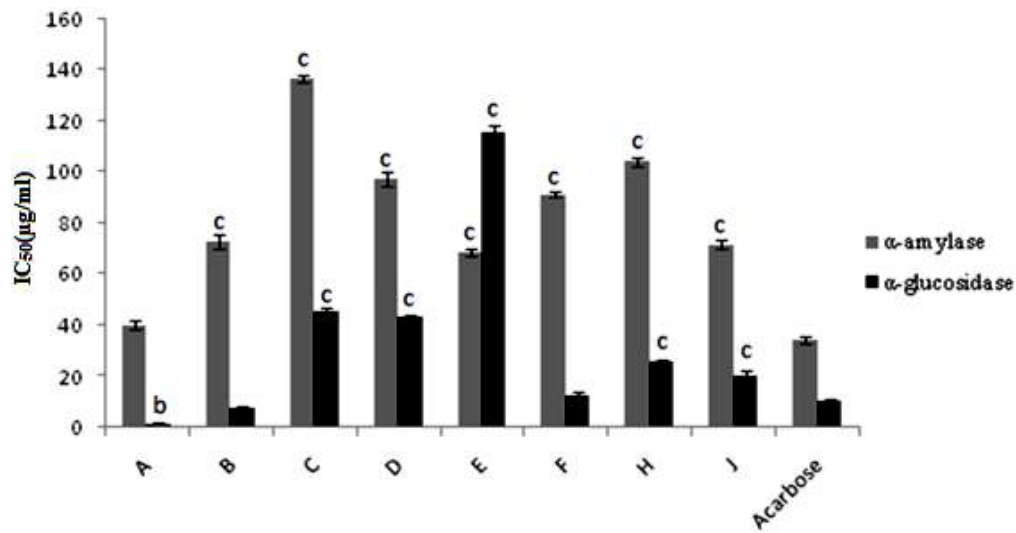
Figure 1. (a) α-Amylase and α-glucosidase inhibitory activity of methanol extracts from Iranian traditional medicinal plants (IC$_{50}$ μg/ml). (b) α-Amylase and α-glucosidase inhibitory activity of ethyl acetate extracts from Iranian traditional medicinal plants (IC$_{50}$ μg/ml). (c) α-Amylase and α-glucosidase inhibitory activity of chloroform extracts from Iranian traditional medicinal plants (IC$_{50}$ μg/ml). (d) α-Amylase and α-glucosidase inhibitory activity of dicholoromethane extracts from Iranian traditional medicinal plants (IC$_{50}$ μg/ml). (e) α-Amylase and α-glucosidase inhibitory activity of *n*-hexane extracts from Iranian traditional medicinal plants (IC$_{50}$ μg/ml). *C. zeylanicum* (A), *C.oxyacantha* (B), *H. sabdariffa* (C), *M. alba* (D), *P. oleracea* (E), *R. fruticosus* (F), *S. aromaticum* (G), *T. polium* (H), *T. graecum* (I), *V. arctostaphylos* (J). a: $p<0.05$ as compared with control. b: $p<0.01$ as compared with control. c: $p<0.001$ as compared with control.

inhibitory effect of ethyl acetate extracts were strongly correlated to phenolic constitutes ($r=0.804$, $p<0.05$), but α-amylase inhibition activity of the extracts were not proportional to total phenolic contents ($r=0.562$).

Chloroform extracts of Tameshk and Kalpoureh show-ed the best results against α-glucosidase with 6.2±0.2

Table 2. Total phenolic content of plants extracts.

Plant species	*n*-Hexane	Dichloromethane	Chloroform	Ethylacetate	Methanol
C. zeylanicum	53.9±0.9	24±1.1	-	99.8±0.8	117.3±1.4
C. oxyacantha	48.1±1.8	16.2±1.1	28.2±0.8	88.6±1.3	82.2±1.9
H. sabdariffa	18.3±1.2	19.2±2.3	34.4±3.1	41.1±1.1	112.1±0.3
M. alba	12.3±0.8	7.3±0.9	14.3±0.5	50.2±2.6	47.3±1.6
P. oleracea	9.3±1.3	6.6±0.8	9.5±1.0	20.3±2.3	22.8±0.9
R. fruticosus	12±1.4	8.9±1.0	27±1.6	77.9±1.4	79.1±0.7
S. aromaticum	136.1±1.0	119.6±1.4	-	-	139.8±0.4
T. polium	13.2±0.8	19.5±1.0	15±1.4	55.8±0.6	62.7±1.4
T. foenum graecum	17.1±1.9	10.7±0.7	14.7±0.9	-	38.4±1.0
V. arctostaphylos	10.8±0.6	26.7±1.6	-	71.5±2.0	100.5±2.1

Each value represents the mean±SD (mg GAE/g of extract).

and 10.2 ±0.4, µg/ml, respectively. It was reported that oral administration of Kalpoureh aqueous extract to a group of streptozotocin diabetic rats for six consecutive weeks, reduced the blood glucose concentration by 64% (Esmaeili and Yazdanparast, 2004). Chloroform extracts of studied plants showed low inhibitory effect against α-amylase with IC_{50} ranged from 60.8 to 160.7 µg/ml. The correlation between total phenolic contents and α-glucosidase and α-amylase inhibition activities in chloroform extracts of studied plants were not significant (r=0.527 and r=0.479, respectively).

Mikhak dichloromethane extract showed the best α-glucosidase and α-amylase inhibitory effect with IC_{50} of 0.8±0.4 and 41.9±0.9 µg/ml, respectively, followed by Darchin dichloromethane extract with IC_{50} of 2.3±0.5 and 46.1±1.0 µg/ml, respectively. Chay-e-makki dichlorome-thane extract showed a moderate α-glucosidase inhibition activity with IC_{50} of 27.3±1.6 µg/ml. Feeding of Chay-e-makki aqueous extract to albine rats, was reported to show hypocholesterolemic and hypoglycemic effect (Agoreyo et al., 2008). There are several reports about *in vivo* hypoglycaemic effect of the related species *Hibiscus rosa-sinensis* (Sachdewa and Khemani, 1999; Sachdewa et al., 2001; Sachdewa and Khemani, 2003). In the dichloromethane extracts, the total phenolic contents and α-glucosidase and α-amylase inhibition potential were significantly correlated (r=0.958, p<0.01 and r=0.741, p<0.05, respectively).

Regarding to α-glucosidase inhibition potential of *n*-hexane extracts, Mikhak and Darchin showed the highest effect (with IC_{50} equal to 1.1±0.3 and 7.0±0.9 µg/ml, respectively), same as methanol and dichloromethane extracts. The same extracts exhibited α-amylase inhibition with IC_{50} of 38.8±1.1 and 50.4±2.7 µg/ml, respectively. According to the Pearson correlation, the total phenolic contents of *n*-hexane extracts were strongly proportional to the α-glucosidase and α-amylase inhibitory activities (r=0.960, p<0.01 and r=0.918, p<0.01, respectively).

It can be seen that in all analyzed extracts, IC_{50} of α-glucosidase inhibition is lower than α-amylase; however in all obtained extracts of Khorfeh, IC_{50} of α-glucosidase inhibition was higher. Previously, Sharma et al. (2009) showed that 50% ethanolic extract of Khorfeh possesses moderate antidiabetic activity on STZ diabetic rats, while exhibiting high antioxidant properties in diabetic conditions which may be due to the presence of flavanoids in the plant. Li et al. (2009) showed that extracted polysaccharide from Khorfeh decreased the concentration of fasting blood glucose (FBG), total cholesterol (TC) and triglyceride (TG) significantly in diabetes mellitus mice.

Despite several studies showing *in vivo* and *in vitro* antidiabetic effect of Shanbalileh aqueous and hydro-alcoholic extracts (Hamden et al., 2010; Nickavar and Yousefian, 2011), analyzed extracts of Shanbalileh showed moderate to weak inhibition effect on α-glucosidase (IC_{50} ranged from 26.7 to 60.2 µg/ml) and α-amylase (IC_{50} ranged from 97.5 to 126.1 µg/ml). This fact may arise from the difference in polarities of the solvents used.

Total phenolic content and antioxidant activity

Total phenolic contents and DPPH radical scavenging-linked antioxidant activities of the plants extracts are shown in Tables 2 and 3, respectively. Total phenolic contents for *n*-hexane extracts ranged from 9.3 to 136.1 mg GAE/g of extract. Mikhak *n*-hexane extract exhibited the highest total phenolic content (136.1±1.0 mg GAE/g of extract), followed by Darchin (53.9±0.9 mg GAE/g of extract) and Sorkhevalik (*Crataegus oxyacantha*) (48.1±1.8 mg GAE/g of extract), whereas all other plants *n*-hexane extracts showed low phenolic contents (<20 mg GAE/g of extract).

The IC_{50} values for DPPH radical scavenging capacity of plants *n*-hexane extracts varied from 0.7 to 183.3

Table 3. DPPH radical scavenging activity (IC_{50} µg/ml) of plant extracts.

Plant species	*n*-Hexane	Dichloromethane	Chloroform	Ethylacetate	Methanol
C. zeylanicum	41.5 ± 0.8^b	49.5 ± 2.2^b	-	16.7 ± 1.0^b	3.1 ± 0.7^b
C. oxyacantha	1.4 ± 0.2^b	9.0 ± 0.5	49.3 ± 3.9^b	7.2 ± 0.3	5.4 ± 0.5
H. sabdariffa	38.7 ± 0.3^b	47.3 ± 1.1^b	36.0 ± 1.4^b	18.0 ± 1.2^b	4.7 ± 0.6^a
M. alba	67.8 ± 0.9^b	56.7 ± 0.9^b	97.9 ± 1.0^b	28.1 ± 0.2^b	20.8 ± 2.1^b
P. oleracea	63.5 ± 1.8^b	91.0 ± 1.5^b	86.0 ± 0.5^b	62.9 ± 2.0^b	85.7 ± 1.6^b
R. fruticosus	76.5 ± 0.5^b	30.1 ± 0.4^b	54.8 ± 2.4^b	35.5 ± 0.2^b	15.2 ± 0.7^b
S. aromaticum	0.7 ± 0.1^b	3.9 ± 0.5^b	-	-	2.2 ± 0.1^b
T. polium	51.8 ± 1.1^b	69.8 ± 0.9^b	71.3 ± 2.1^b	20.8 ± 2.5^b	4.4 ± 0.3^a
T. foenum graecum	183.3 ± 2.3^b	275.0 ± 1.1^b	184.4 ± 4.1^b	-	161.5 ± 2.5^b
V. arctostaphylos	136.5 ± 1.3^b	46.9 ± 0.7^b	-	10.6 ± 0.5	7.9 ± 0.6
BHT	9.2 ± 0.4				

Each value represents the mean±SD, statistically significance compared with control (a: $^p<0.01$ and ^{b}: $p<0.001$).

µg/ml. IC_{50} is the required concentration of the plant extract to scavenge 50% of DPPH radical in the reaction mixture under the experimental conditions. *n*-Hexane extract of Mikhak had the highest activity against DPPH (IC_{50} at 0.7 ± 0.1 µg/ml), followed by Sorkhevalik (IC_{50} at 1.4 ± 0.2 µg/ml), whereas *n*-hexane extracts of Shanbalileh and Qareqat showed the lowest antioxidant activity with IC_{50} equal to 183.3 ± 2.3 and 136.5 ± 1.3 µg/ml, respectively. In *n*-hexane extracts, DPPH inhibitory effect and total phenolic contents were strongly correlated ($r=0.923$, $p<0.01$). Also the correlation between DPPH radical scavenging activity and α-amylase inhibitory effect was significant ($r=0.781$, $p<0.001$), while no correlation was observed with α-glucosidase inhibition activity ($r=0.236$).

Many studies reported the antioxidant activity of Mikhak essential oil and its non-polar extract. The ability of Mikhak essential oil to scavenge free radicals generated during aflatoxicosis in rats has been demonstrated and it has been associated with the phenolic compounds present in the oil (Abdel-Wahhab and Aly, 2005). Lee and Shibamoto (2001) investigated the antioxidant activity of Mikhak aroma extract and its major aroma components, eugenol and eugenol acetate, and found it comparable to that of α-tocopherol (vitamin E), as a natural antioxidant. Arung et al. (2011) evaluated the effect of methanolic extract and essential oil of Mikhak bud on melanin formation in B16 melanoma cells. They isolated eugenol and eugenol acetate as the active compounds.

The total phenolic and antioxidant activity of dichloromethane and chloroform extracts of studied plants except dichloromethane extract of Mikhak were insignificant. Dichloromethane extract of Mikhak exerted high total phenolic content (119.6 ± 1.4 mg GAE/g of extract) and DPPH radical scavenging activity (IC_{50} at 3.9 ± 0.5 µg/ml). Dichloromethane extract of Khorfeh (*P. oleracea*) showed the lowest total phenolic content

(6.6 ± 0.9 mg GAE/g of extract), not only among dichloromethane and chloroform extracts but also within all analyzed extracts. The correlations between antioxidant activities and total phenolic contents of dichloromethane and chloroform extracts were significant ($r=0.909$, $p<0.01$ and $r=0.891$, $p<0.01$, respectively). Additionally, the correlation between antioxidant activity and α-glucosidase and α-amylase inhibitory effects of dichloromethane extracts were moderate, but statistically significant ($r=0.401$, $p<0.05$ and $r=0.410$, $p<0.05$, respectively). Regarding to the chloroform extracts, there were no correlation between DPPH radical scavenging-linked antioxidant activity and α-glucosidase and α-amylase inhibitory effects ($r=0.162$ and $r=0.308$, respectively).

It can be seen that the more polar extracts of plants such as methanol and ethylacetate extracts showed higher phenolic content than non-polar extracts.

Among ethyl acetate extracts, Sorkhevalik, with 88.6 ± 1.3 mg GAE/g of extract total phenolic content possessed the highest antioxidant activity (IC_{50} at 7.2 ± 0.3 µg/ml). Darchin ethyl acetate extract exhibited the highest total phenolic content (99.8 ± 0.8 mg GAE/g of extract), but not high antioxidant activity with IC_{50} at 16.7 ± 1.0 µg/ml. Antioxidant activity and total phenolic content for ethyl acetate extracts were moderately significant ($r=0.575$). Khorfeh ethyl acetate extract showed the lowest total phenolic content (20.3 ± 2.3 mg GAE/g of extract) linked to antioxidant activity (IC_{50} at 62.9 ± 2.0 µg/ml) in this group. The correlation between DPPH radical scavenging activity and α-glucosidase inhibition of ethyl acetate extracts was strongly proportional ($r=0.833$, $p<0.001$), whereas a moderate correlation was observed with the α-amylasa inhibition activity ($r=0.419$, $p<0.05$).

Methanolic extracts of the plants had the highest phenolic contents in comparison with other extracts. Four methanolic extracts showed very high phenolic contents

(over 100 mg GAE/g of extract), that is, Mikhak, Darchin, Chay-e-makki (*H. sabdariffa*) and Qaraqat with 139.8±0.4, 117.3±1.4, 112.1±0.3 and 100.5±2.1 mg GAE/g of extract, respectively. Significant correlation were found between antioxidant potential and total phenolic contents of methanolic extracts (r=0.858, $p<$ 0.01). Mikhak and Darchin methanolic extracts showed highest DPPH radical scavenging capacity with IC_{50} of 2.2±0.1 and 3.1±0.7 µg/ml, followed by Kalpoureh (*T. polium*), Chay-e-makki, Sorkhevalik and Qareqat methanolic extracts, with IC_{50} of 4.4±0.3, 4.7±0.6, 5.4±0.5 and 7.9±0.6 µg/ml, respectively. All mentioned extracts were stronger antioxidants than BHT as a commercial and synthetic antioxidant which exhibited IC_{50} equal to 9.2±0.4 µg/ml. Methanol extract of Shanbalileh possessed the lowest DPPH radical inhibition activity with IC_{50} at 161.5±2.5 µg/ml. According to the Pearson correlation results; DPPH linked antioxidant activity of methanolic extracts were significantly proportional to the α-glucosidase and α-amylase inhibitory activities (r=0.647, $p<0.001$ and r=0.842, $p<0.001$, respectively).

Conclusion

In the present study, the inhibition ability of 45 various extracts obtained from 10 traditionally used medicinal plants in Iran against diabetes mellitus for inhibition of α-glucosidase and α-amylase, key enzymes relevant to type 2 diabetes, were investigated. The obtained results highlight the high activities of Mikhak and Darchin extracts and provide some scientific support to their traditional use. We conclude that further bioassay-guided fractionation approaches will be required on these species to identify the compounds responsible for their promising *in vitro* anti-diabetic activity.

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