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Original Article

Antioxidant and antidiabetic activities of 11 herbal plants from Hyrcania region, Iran

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ABSTRACT

In the present study, 2,2-diphenyl-1-picrylhydrazyl radical scavenging, α-amylase and α-glucosidase inhibition activities, and total phenolic contents of *n*-hexane, ethyl acetate, and methanol extracts of various parts of *Allium paradoxum*, *Buxus hyrcana*, *Convolvulus persicus*, *Eryngium caucasicum*, *Heracleum persicum*, *Pimpinella affinis*, *Parrotia persica*, *Primula heterochroma*, *Pyrus boissieriana*, *Ruscus hyrcanus*, and *Smilax excelsa* were investigated. These plants, which mostly serve as food flavoring, were collected from Hyrcania region, Sari, Iran. Some extracts of *H. persicum*, *S. excels*, *P. boissieriana*, *P. persica*, and *P. heterochroma* exhibited significant antidiabetic activities in α-amylase and α-glucosidase assays, more effective than acarbose (concentrations that cause 50% inhibition = 75.7 μg/mL and 6.1 μg/mL against α-amylase and α-glucosidase, respectively). Also, *C. persicus*, *P. boissieriana*, and *P. heterochroma* showed strong antioxidant activities, compared with butylated hydroxytoluene (concentration that causes 50% inhibition = 16.7 μg/mL). In conclusion, this study can recommend these plants as good candidates for further investigations to find potent antidiabetic natural products or probable lead compounds. Statistical analysis showed significant correlation between the 2,2-diphenyl-1-picrylhydrazyl scavenging activity and total phenolic contents ($r = 0.711$, $p < 0.001$).

1. Introduction

Plants have developed an array of defense strategies (antioxidant systems) to manage oxidative stress. In these systems, there is a wide variety of antioxidants [e.g., ascorbic acid, gluthione, uric acid, tocopherol, carotenoids, and (poly)phenols], which are different in their composition, mechanism, and site of action [1]. Antioxidants have significant inhibition roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases such as inflammation, cancer, and atherosclerosis [2]. Moreover, having antioxidant activity in addition to pharmaceutical properties, such as antidiabetic, anticarcinogenic, and antialzheimeric activities, can be a special function to obtain multifunctional drugs. Recently,

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there has been an increased interest globally to discover natural antioxidants with low or no side effects for use in preventive medicine and the food industry [3].

Noninsulin-dependent diabetes mellitus or type-II diabetes mellitus is one of the most common and serious metabolic disorders with abnormally high blood glucose levels (hyperglycaemia) due to defects in insulin secretion, or action, or both [4]. Hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. Because α-glucosidase and pancreatic α-amylase play a critical role in carbohydrate digestion and glycoprotein processing, inhibitors of these enzymes might be used to treat diabetes, human immunodeficiency virus, Gaucher's disease, cancers, and Alzheimer's disease [5–7]. Some inhibitors, such as acarbose, miglitol (a deoxynojirimycin derivative), and voglibose, are widely used clinically in combination with diet to control blood glucose levels of patients [8,9]. To prevent or decline the side effects of these drugs and also to provide more candidates of drug choices, it is still essential to seek new α-glucosidase inhibitors for further drug development. In recent years, many efforts have been made to approach glucosidase inhibitors from natural sources for antidiabetes treatment [10,11].

One of the major hypotheses proposed to explain the hyperglycaemia-induced onset of diabetic complications is that it is a result of the impairment in the equilibrium between reactive oxygen species capacity and antioxidant defence capacity [12–14]. Accordingly, using antioxidant agents can be helpful for scavenging various reactive oxygen species and prevention of diabetes mellitus.

In this work, antioxidant and antidiabetic activities of different extracts of *Allium paradoxum* (M.B.) G.Don (Liliaceae), *Buxus hyrcana* Pojark. (Buxaceae), *Convolvulus persicus* L. (Convolvulaceae), *Eryngium caucasicum* Trautv. (Apiaceae), *Heracleum persicum* Desf. ex Fischer (Apiaceae), *Pimpinella affinis* Ledeb. (Apiaceae), *Parrotia persica* C.A. Mey (Hamamelidaceae), *Primula heterochroma* Stapf (Primulaceae), *Pyrus boissieriana* Buhse (Rosaceae), *Ruscus hyrcanus* Woron. (Asparagaceae), and *Smilax excelsa* L. (Smilacaceae) were investigated. These plants were collected in Sari, Hyrcania region, Iran. The Hyrcania (Caspian) region, that covers an area of 1,925,125 ha, extends throughout the south coast of the Caspian Sea in the northern part of Iran [15]. In this region, people use these plants as food flavoring, and antiflatulence, antimicrobial, antifever, and antidiabetic natural sources (Table 1).

Antioxidant activities of *n*-hexane, ethyl acetate, and methanol extracts of various parts of these plants were examined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Also, the total phenolic content of these herbal plants was determined. In addition, inhibition activities of the extracts against pancreatic α-amylase and α-glucosidase were investigated.

2. Methods

2.1. Reagents

All the chemicals were purchased from Sigma–Aldrich Chemie Gmbh (Munich, Germany) and Merck (Darmstadt, Germany) companies. The chemicals were of analytical grades.

2.2. Plant materials

Different parts of *A.paradoxum*, *B. hyrcana*, *C. persicus*, *E. caucasicum*, *H. persicum*, *P. affinis*, *P. persica*, *P. heterochroma*, *P. boissieriana*, *R. hyrcanus*, and *S. excelsa* were collected from lowland to submountain forest areas of Sari, Mazandaran province, Iran in April and May 2011. Voucher specimens were deposited in the herbarium of Nowshahr botanical garden, Nowshahr, Iran (Table 1). The plant materials were dried at room temperature and ground to a powder in a blender.

2.3. Solvent extraction of the plants

The protocol for extraction of the plants was sequential extraction using three different solvents with different polarities, starting with the most nonpolar. The dried and fine plant parts (100 g) were extracted with 400 mL *n*-hexane by maceration (48 hours × 2). By addition of 400 mL ethyl acetate to the dried plant's surplus, ethyl acetate extract was obtained (48 hours × 2). The methanol extract was obtained by the same method (48 hours × 1). Each extract was then concentrated under reduced pressure at approximately 40°C to obtain *n*-hexane, ethyl acetate, and methanol fractions. The percentage of extract yield was calculated as (dry extract weight/dry starting material weight) × 100 (Table 2).

2.4. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The ability of plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined according to the method described by Chiu et al [44]. A 50-μL aliquot of 500 μg/mL of test sample in dimethyl sulfoxide (DMSO) was added to 200 μL of 100μM DPPH solution in methanol. After an incubation period of 30 minutes at room temperature in the darkness, the decrease in the absorbance (Abs) was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive control for this assay. Experiments were carried out three times. The percentage inhibition was calculated using the following equation:

$$\%\text{Inhibition} = \frac{(\text{Abs}_{517}(\text{Control}) - \text{Abs}_{517}(\text{Sample}))}{\text{Abs}_{517}(\text{Control})} \times 100$$

DPPH scavenging activities of various concentrations of the most effective extracts were assessed to determine concentration that causes 50% inhibition (IC_{50}).

2.5. Determination of total phenolic contents

Total phenolic contents of the extracts were determined using the Folin–Ciocalteu method [45]. A 2.5-μL sample of each extract (1000 μg/mL in DMSO) was added to 12.5 μL of Folin–Ciocalteu reagent and 195 μL of phosphate buffer (75mM, pH 7.0). After 3 minutes, 50 μL of Na_2CO_3 (7.0%) was added and incubated at room temperature for 2 hours. After 2 hours the absorbance was measured at 765 nm. Standard curve was prepared by using different concentrations of gallic

Table 1 – Plant species, their traditional uses, and biological properties.

Plant species	Medicinal properties	Traditional uses	Voucher No.[a]	Family
Allium paradoxum	Antihemolytic [17], antioxidant [18], protective against gentamicin-induced nephrotoxicity [19]	Food flavoring, antiacne, antidigestive disorders [16]	2961	Liliaceae
Buxus hyrcana	Anti-plasmodial activity [20], acetylcholinesterase-inhibitor [21,22], immunosuppressive [23], antioxidant, anti-HIV [24], antifungal [25]	Antimalaria, antipneumonia, antihair loss, antirheumatism, laxative, febrifuge, anti-infection, analgesic, antiheadache, antiepileptic, aperient [16,20]	1529	Buxaceae
Convolvulus persicus	—	—	6005	Convolvulaceae
Eryngium caucasicum	Antioxidant [28]	Food flavoring [16,26,27]	6159	Apiaceae
Heracleum persicum	Anticonvulsant [32], antitumor, antibacterial [31], antifungal [34], anti-inflammatory, analgesic [35], antioxidant [36,37], cytotoxic [38]	Food flavoring [16,29], analgesic [30], antiseptic [31], antiepilepsy [32], antimicrobial [33], antiflatulence, antidyspepsia [16]	9869	Apiaceae
Pimpinella affinis	—	Food flavoring, antispasmodic, narcotic, expectorant, diuretic, antimigraine, antimicrobial, antiasthma, carminative, anti-cholera [16]	3148	Apiaceae
Parrotia persica	Antioxidant [39], antibacterial [40]	Food coloring and food flavoring, antifever [39,40]	8370	Hamamelidaceae
Primula heterochroma	Antihemolytic [41]	Food flavoring [16]	3136	Primulaceae
Pyrus boissieriana	Antioxidant [24]	Food flavoring, anti-infection, narcotic, anticramp, antihypertensive [16]	4607	Rosaceae
Ruscus hyrcanus	—	Diuretic, appetizer, antilaxative, vasoconstrictor, antibleeding, antinephritis, anti-infection, aperient, antivaricose, laxative [16]	9407	Asparagaceae
Smilax excelsa	Antioxidant [42], cytotoxic, antimicrobial [43]	Food flavoring, diuretic, sudatory, antieczema [16,42]	2973	Smilacaceae

[a] Voucher specimens were deposited in the herbarium of Nowshahr botanical garden, Nowshahr, Iran.

acid. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g dry weight. Analyses were done in triplicate.

2.6. Pancreatic α-amylase inhibition assay

The pancreatic α-amylase inhibition assay was performed according to the literature procedure [46]. Briefly, 50 μL of samples (1000 μg/mL in DMSO) were added to 150 μL starch solution (containing 1% starch and 17mM NaCl). The reaction was initiated by adding 10 μL α-amylase (26 U/mL) to the reaction mixture. After 30 minutes, the reaction was stopped by adding 20 μL of NaOH solution (2N). Subsequently, 20 μL of dinitrosalicylic acid reagent (44mM 3,5-dinitrosalicylic acid, 106mM potassium sodium tartarate, 40mM NaOH) was added to the reaction mixture. The mixtures were heated at 100°C for 20 minutes. After cooling to room temperature, Abs was recorded at 540 nm using a spectrophotometer. Standard curve was prepared by using different concentrations of maltose after addition of dinitrosalicylic acid reagent to determine equal absorption of the produced maltose. Acarbose was used as a positive control for this assay. All samples were analyzed in triplicate. The percentage inhibition was calculated using follow the equation.

$$\%\text{Inhibition} = \frac{[\text{Abs}_{540}(\text{Control}) - \text{Abs}_{540}(\text{Sample})]}{\text{Abs}_{540}(\text{Control})} \times 100$$

In this equation, Abs_{540} (sample) is the absorption of maltose produced from starch by the enzyme at 540 nm in the presence of the extract, and Abs_{540} (control) is the equal absorption of the produced maltose by the enzyme at 540 nm in the absence of the extract.

α-Amylase inhibitory activities of various concentrations of the most effective extracts were assessed to determine IC_{50}.

2.7. α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was performed according to the literature procedure [47]. A 40-μL aliquot of α-glucosidase solution (1 U/mL) was added to 1 μL of sample solution (500 μg/mL in DMSO) and 69 μL of 0.1M sodium phosphate buffer (pH = 7.0). After 15 minutes' incubation at 37°C, 40 μL of substrate solution (5mM p-nitrophenyl α-D-glucopyranoside) was added to the reaction mixture and incubated at 37°C for 30 minutes. Then, the reaction terminated by adding 150 μL of

Table 2 – Results of extraction yields, DPPH radical scavenging activity, total phenolic contents, and α-amylase and α-glucosidase inhibition activity of the medicinal plants.

	Plant species	Plant part used	Extract yield (%W/W)		DPPH scavenging activity (%)[a]	Total phenolic content (mg GAE/g DW)	α-Amylase inhibition (%)[b]	α-Glucosidase inhibition (%)[c]
1	*Allium paradoxum*	Aerial parts	Hex.	1.2	27.6 ± 0.7^{d}	23.0 ± 1.4	45.9 ± 5.5^{d}	15.3 ± 1.3^{d}
			EA.	1.9	22.3 ± 0.3^{d}	53.0 ± 3.1	17.8 ± 2.0^{f}	NI
			Met.	4.0	47.1 ± 0.3^{d}	85.6 ± 2.9	33.5 ± 5.0^{d}	27.8 ± .06^{d}
		Bulb	Hex.	0.3	43.7 ± 0.7^{d}	39.6 ± 4.5	36.8 ± 1.6^{f}	37.7 ± 1.3^{e}
			EA.	0.3	68.3 ± 0.1^{d,*}	118.7 ± 6.3	12.9 ± 3.4^{d}	9.2 ± 1.5^{d}
			Met.	1.8	41.6 ± 0.4^{d}	110.1 ± 4.1	NI	NI
2	*Buxus hyrcana*	Leaf	Hex.	1.9	20.8 ± 0.6^{d}	15.3 ± 3.3	15.2 ± 2. 1^{e}	42.9 ± 2.3^{e}
			EA.	1.6	33.8 ± 0.1^{d}	101.6 ± 5.1	NI	NI
			Met.	5.4	47.8 ± 0.8^{d}	148.2 ± 4.1	33.3 ± 2.0^{e}	12.3 ± 1.1^{e}
3	*Convolvulus persicus*	Aerial parts	Hex.	1.8	19.4 ± 1.1^{d}	39.0 ± 4.7	4.7 ± 2.2^{f}	NI
			EA.	2.2	37.9 ± 0.8^{e}	55.2 ± 2.8	8.7 ± 1.0^{e}	NI
			Met.	5.1	88.3 ± 0.6^{d,*}	134.7 ± 6.2	NI	NI
		Root	Hex.	0.7	48.8 ± 0.8^{d}	65.4 ± 3.2	17.7 ± 3.7^{d}	2.9 ± 1.0^{d}
			EA.	1.6	56.6 ± 1.4^{d,*}	77.0 ± 2.2	42.9 ± 0.5^{d}	13.0 ± 1.5^{d}
			Met.	7.1	55.3 ± 0.9^{d,*}	81.9 ± 4.2	20.6 ± 3.1^{e}	6.7 ± 2.0^{e}
4	*Eryngium caucasicum*	Aerial parts	Hex.	1.0	29.7 ± 0.7^{d}	7.1 ± 0.6	9.6 ± 2.7^{f}	NI
			EA.	1.8	24.4 ± 1.7^{d}	14.4 ± 3.3	11.9 ± 1.3^{e}	NI
			Met.	5.0	31.2 ± 2.1^{d}	86.2 ± 5.0	15.9 ± 4.8^{f}	NI
5	*Heracleum persicum*	Aerial parts	Hex.	1.4	26.2 ± 0.4^{d}	42.0 ± 1.7	78.5 ± 3.9^{d,*}	66.4 ± 1.6^{d,*}
			EA.	1.5	76.6 ± 0.7^{d,*}	167.2 ± 2.3*	38.9 ± 1.0^{e}	3.8 ± 1.3^{f}
			Met.	3.4	41.5 ± 0.3^{d}	113.2 ± 5.8	30.8 ± 5.0^{f}	NI
		Root	Hex.	2.1	5.2 ± 0.4^{d}	58.9 ± 1.7	41.9 ± 2.7^{e}	84.5 ± 1.2^{e,*}
			EA.	1.9	28.7 ± 0.6^{e}	90.8 ± 4.4	10.5 ± 3.3^{e}	NI
			Met.	4.2	19.1 ± 0.1^{d}	78.2 ± 5.1	NI	NI
6	*Pimpinella affinis*	Leaf	Hex.	1.2	29.0 ± 0.6^{d}	31.0 ± 3.6	81.3 ± 3.7^{d,*}	26.4 ± 1.3^{e}
			EA.	1.0	31.6 ± 0.9^{d}	54.3 ± 2.1	26.2 ± 2.4^{f}	NI
			Met.	2.6	91.5 ± 1.2^{d,*}	155.5 ± 5.5*	24.5 ± 3.0^{e}	0.3 ± 2.1^{f}
		Root	Hex.	1.3	11.5 ± 0.6^{d}	20.6 ± 4.4	5.5 ± 0.6^{f}	33.6 ± 3.3^{e}
			EA.	2.2	36.2 ± 0.6^{d}	37.1 ± 3.3	76.4 ± 1.0^{e,*}	3.5 ± 1.0^{a}
			Met.	3.8	32.3 ± 0.9^{e}	90.5 ± 3.5	27.6 ± 5.1^{f}	NI
7	*Parrotia. persica*	Leaf	Hex.	0.4	27.5 ± 1.3^{d}	37.7 ± 2.5	25.6 ± 2.3^{e}	35.7 ± 0.4^{e}
			EA.	0.6	96.0 ± 1.0^{d,*}	145.7 ± 8.3	45.6 ± 4.7^{e}	99.3 ± 2.0^{d,*}
			Met.	5.1	95.9 ± 0.7^{d,*}	506.5 ± 11.3*	38.3 ± 1.4^{e}	99.6 ± 2.8^{d,*}

Table 2 – (continued)

	Plant species	Plant part used	Extract yield (%W/W)		DPPH scavenging activity (%)[a]	Total phenolic content (mg GAE/g DW)	α-Amylase inhibition (%)[b]	α-Glucosidase inhibition (%)[c]
8	*Primula. heterochroma*	Leaf	Hex.	0.5	15.4 ± 0.3[d]	24.9 ± 5.8	14.7 ± 1.3[e]	NI
			EA.	0.8	39.9 ± 0.8[d]	82.5 ± 1.3	37.6 ± 4.3[e]	NI
			Met.	4.3	95.5 ± 0.7[d,*]	223.7 ± 5.0*	31.0 ± 3.9[a]	97.8 ± 1.9[d,*]
		Root	Hex.	0.4	13.7 ± 1.4[d]	32.8 ± 2.8	NI	NI
			EA.	0.8	56.5 ± 0.5[d,*]	153.4 ± 4.5*	4.5 ± 2.9[f]	61.8 ± 1.2[d,*]
			Met.	5.7	95.1 ± 0.8[d,*]	165.7 ± 6.3*	12.7 ± 2.1[e]	98.7 ± 3.3[d,*]
9	*Pyrus boissieriana*	Leaf	Hex.	0.9	27.8 ± 0.7[d]	21.8 ± 5.1	1.6 ± 3.1[e]	20.4 ± 1.0[e]
			EA.	1.6	84.5 ± 0.5[d,*]	174.6 ± 3.8*	2.0 ± 1.1[d]	NI
			Met.	3.5	94.3 ± 0.4[d,*]	414.5 ± 9.5*	30.2 ± 4.0[d]	51.6 ± 1.3[e,*]
		Stem	Hex.	0.3	25.1 ± 1.1[e]	15.7 ± 4.0	44.1 ± 2.5[f]	99.2 ± 4.1[d,*]
			EA.	0.6	94.0 ± 1.7[d,*]	312.6 ± 6.4*	25.2 ± 1.0[d]	93.4 ± 1.6[d,*]
			Met.	3.8	94.7 ± 0.4[d,*]	549.5 ± 8.3*	56.5 ± 4.4[e,*]	99.1 ± 2.6[d,*]
10	*Ruscus hyrcanus*	Aerial parts	Hex.	0.7	19.2 ± 0.9[d]	12.6 ± 2.3	13.9 ± 3.2[e]	19.1 ± 1.3[e]
			EA.	0.8	32.4 ± 0.7[d]	94.2 ± 4.2	20.4 ± 1.8[e]	NI
			Met.	3.5	25.0 ± 2.4[d]	83.1 ± 2.7	8.8 ± 1.3[d]	7.3 ± 0.6[e]
		Root	Hex.	0.2	4.8 ± 0.5[d]	57.1 ± 3.3	15.3 ± 1.0[f]	30.1 ± 1.3[d]
			EA.	0.5	35.3 ± 0.8[d]	170.9 ± 5.2*	1.6 ± 2.1[f]	NI
			Met.	6.8	10.5 ± 0.5[e]	42.6 ± 3.4	10.8 ± 4.2[f]	NI
11	*Smilax excelsa*	Leaf	Hex.	1.2	22.1 ± 0.3[d]	19.3 ± 2.7	78.2 ± 3.0[d,*]	15.1 ± 1.0[e]
			EA.	1.3	22.4 ± 1.4[d]	97.9 ± 3.5	76.1 ± 4.6[d,*]	2.7 ± 1.1[d]
			Met.	3.9	47.1 ± 0.3[d]	239.0 ± 5.4*	98.5 ± 3.3[e,*]	1.5 ± 1.4[d]
		Stem	Hex.	0.7	43.7 ± 0.7[e]	7.1 ± 2.1	10.8 ± 2.9[f]	29.6 ± 2.2[d]
			EA.	1.0	68.3 ± 0.8[d,*]	134.1 ± 6.5	98.5 ± 3.1[e,*]	58.9 ± 0.4[e,*]
			Met.	2.9	41.7 ± 0.7[d]	226.7 ± 4.3*	38.5 ± 1.7[d]	32.1 ± 2.4[f]
	BHT	—	—	—	99.6 ± 1.1[g]	—	—	—
	Acarbose	—	—	—	—	—	74.9 ± 1.3[h]	41.7 ± 0.7[i]

The extracts were tested at concentrations of [a]200 μg/mL for DPPH scavenging, [b]238.1 μg/mL for α-amylase inhibitory, and [c]3.3 μg/mL for α-glucosidase inhibitory assays.
Values are presented as the mean ± standard deviation of three independent experiments. [d] $p < 0.001$, [e] $p < 0.01$, [f] $p < 0.05$ as compared with control.
*The best results.
BHT = butylated hydroxytoluene; DPPH = 2,2-diphenyl-1-picrylhydrazyl; DW = dry weight; EA = ethyl acetate; GAE = gallic acid equivalent; Hex. = *n*-hexane; Met. = methanol; NI = not identified.

0.1M Na_2CO_3. The Abs was determined at 405 nm using a spectrophotometer and the percentage inhibition was calculated using the following equation. Acarbose was used as a positive control for this assay. All samples were analyzed in triplicate.

$$\%\text{Inhibition} = \frac{(\text{Abs}_{405}(\text{Control}) - \text{Abs}_{405}(\text{Sample}))}{\text{Abs}_{405}(\text{Control})} \times 100$$

In this equation, Abs_{405} (sample) is the absorption of the produced *p*-nitrophenol from *p*-nitrophenyl α-D-glucopyranoside by the enzyme in 405 nm in the presence of the extract, and Abs_{405}(control) is the absorption of produced *p*-nitrophenol by the enzyme in 405 nm in the absence of the extract.

α-Glucosidase inhibitory activities of various concentrations of the most effective extracts were assessed to determine IC_{50}.

2.8. Statistical analysis

All assays were performed at least in triplicate and the data were expressed as mean ± standard deviation. Statistical analyses were carried out using SPSS version 19.0 (SPSS Inc., Armonk, NY, USA). One-way analysis of variance followed by Tukey's multicomparison test was used for comparing the results among treatments. Differences were considered significant at $p < 0.01$. IC_{50} values were determined by plotting a percent of inhibition versus concentration curve for positive controls in α-amylase, α-glucosidase, and DPPH radical scavenging assays.

3. Results

The extraction yield from 11 aromatic plants by three solvents (57 extracts of *n*-hexane, ethyl acetate, and methanol) is represented in Table 2.

The present study was designed to investigate the bioactive properties of the aforementioned plants. These properties included DPPH radical scavenging activity, total phenolic contents, and also α-amylase and α-glucosidase inhibition activity. The results of these assays are shown in Table 2, in which the best are marked with asterisks (*).

The DPPH radical scavenging assay measures the reduction of DPPH radical by hydrogen-donating or electron-transferring antioxidants due to the formation of the non-radical form, DPPH-H. The extracts were tested at a concentration of 200 μg/mL. The results showed that ethyl acetate and methanol extracts of leaves of *P. persica* (96.0% and 95.9%, respectively), methanol extract of leaves of *P. affinis* (91.5%), methanol extracts of leaves and roots of *P. heterochroma* (95.5%

and 95.1%, respectively) and ethyl acetate and methanol extracts of leaves and stems of *P. boissieriana* (84.5%, 94.3%, 94.0%, and 94.7%, respectively) exhibited strong antioxidant activities. Generally, ethyl acetate and methanol extracts of *P. boissieriana* and *P. heterochroma* had the best antioxidant activities (Table 2). BHT was used as standard antioxidant (99.6%).

According to the total phenolic assay, methanol extracts of *P. persica*, *P. heterochroma*, *P. boissieriana*, and *S. excelsa* were rich in phenolic compounds. It can be concluded that highly polar solvents are more effective in extracting phenolic compounds from plant materials than the less polar solvents, as has already been reported [48,49]. The best results are marked with asterisks (*) in Table 2.

The maltose standard curve for α-amylase inhibitory assay was plotted using various concentrations of maltose (Fig. 1). The extracts were tested at a concentration of 238.1 μg/mL for inhibition of α-amylase. A significant inhibition was observed with *n*-hexane, ethyl acetate, and methanol extracts of leaves of *S. excelsa* (78.2%, 76.1%, and 98.5%, respectively). Also, ethyl acetate extract of stems of *S. excelsa* was found to have high inhibition activity (98.5%). *n*-Hexane extracts of *P. affinis* (leaves) and *H. persicum* (aerial parts) showed enzyme inhibition of 81.3% and 78.5%, respectively. Some of the plant extracts including *n*-hexane extract of *P. heterochroma*, ethyl acetate extract of *B. hyrcana,* and methanol extracts of *A. paradoxum*, *C. persicus*, and *H. persicum* showed negative values, which indicates that no inhibition occurred at 238.1 μg/mL. In this assay, the positive control, acarbose, showed a 74.9% inhibitory effect (Table 2).

α-Glucosidase inhibitory activities of the plants were assessed using 3.3 μg/mL of the different extracts. High levels of α-glucosidase inhibition were observed in *n*-hexane, ethyl acetate, and methanol extracts of stems of *P. boissieriana* (99.2%, 93.4% and 99.1%, respectively). Also, ethyl acetate and methanol extracts of *P. persica* and *P. heterochroma* was found to have high inhibitory activity (99.3%, 99.6%, 61.8%, and 98.7%, respectively). The results were compared with those of acarbose (41.7%; Table 2).

The statistical analysis showed significant correlation between α-amylase and α-glucosidase inhibitory activities ($r = 0.335$, $p < 0.01$) and also between DPPH scavenging activity and total phenolic contents ($r = 0.711$, $p < 0.001$) for all of the extracts. There is a good correlation between α-glucosidase inhibitory activity and DPPH scavenging activity ($r = 0.381$, $p < 0.01$), and also between α-glucosidase inhibitory activity and total phenolic contents ($r = 0.422$, $p < 0.001$). However, there were no significant correlations between α-amylase inhibitory activity with DPPH radical scavenging ($r = 0.223$, $p < 0.1$) and total phenolic contents ($r = 0.229$, $p < 0.1$) [50,51]. All the correlation curves are shown in Fig. 2.

The IC_{50} values of the most effective extracts in DPPH scavenging, α-amylase and α-glucosidase assays, are shown in Table 3. As indicated, all the plant extracts exhibited lower DPPH scavenging activity than BHT. Among them, methanol extract of *C. persicus* roots (IC_{50} = 38.9 μg/mL), methanol extract of *P. heterochroma* leaves, and ethyl acetate and methanol extracts of its roots (IC_{50} = 41.7 μg/mL, 37.9 μg/mL, and 30.1 μg/mL, respectively), and methanol extract of *P. boissieriana* stems (IC_{50} = 39.3 μg/mL) showed better antioxidant activities. All these extracts exhibited high concentration-dependent activity in the DPPH scavenging assay.

As shown in Table 3, the *n*-hexane extracts of *H. persicum* aerial parts and roots and the ethyl acetate extract of *S. excelsa*

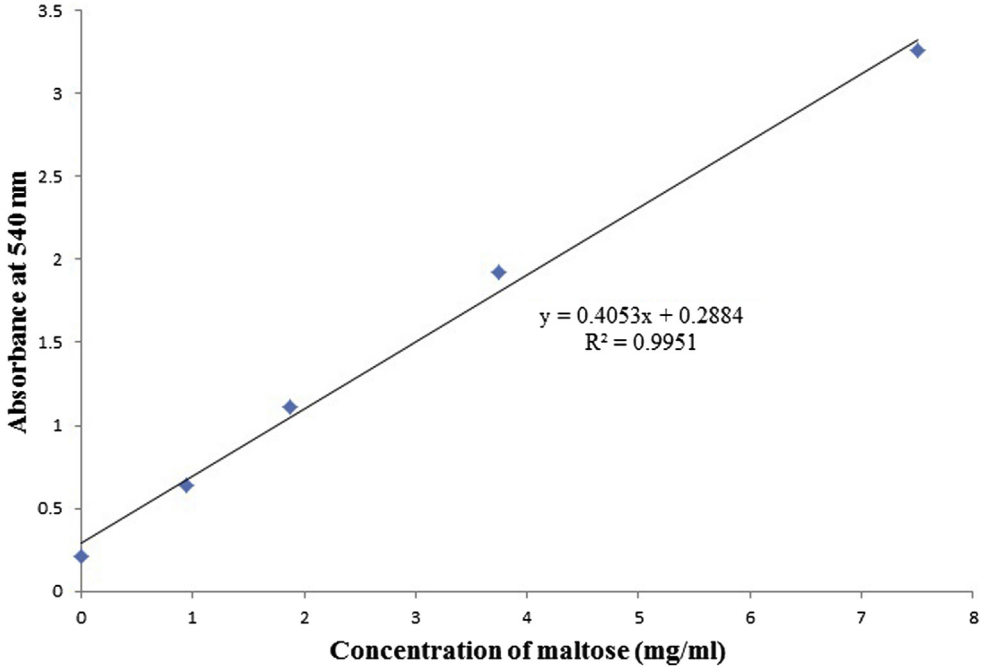

Fig. 1 – Maltose standard curve for α-amylase inhibitory assay.

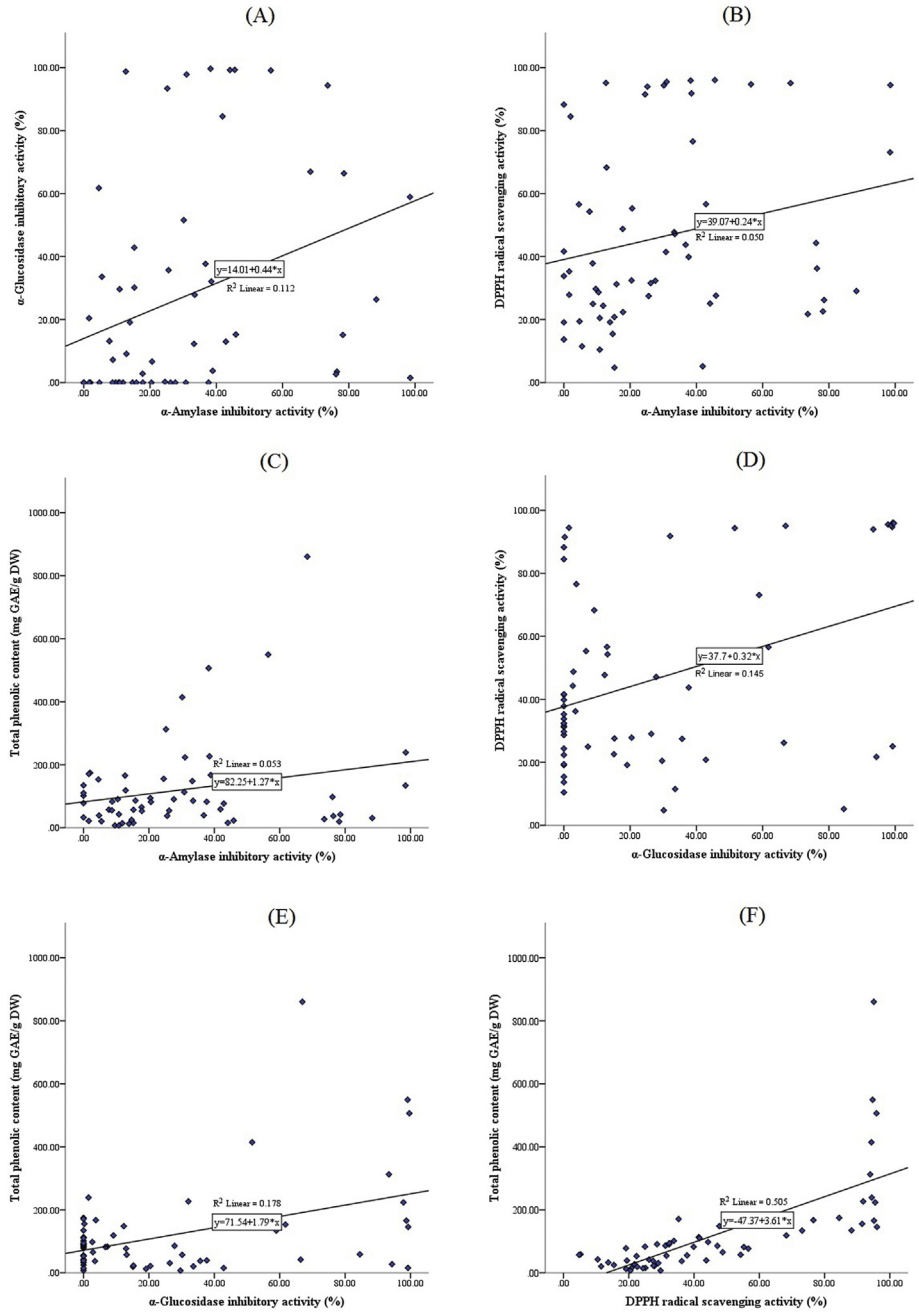

Fig. 2 – Correlations between: (A) α-amylase and α-glucosidase inhibitory activities; (B) α-amylase inhibitory activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity; (C) α-amylase inhibitory activity and total phenolic contents; (D) α-glucosidase inhibitory activity and DPPH radical scavenging activity; (E) α-glucosidase inhibitory activity and total phenolic contents; and (F) DPPH radical scavenging activity and total phenolic contents.

stems exhibited significant inhibitory activities against α-amylase and α-glucosidase, even more effective than acarbose. Also, the methanol extract of *P. boissieriana* leaves and all the extracts of its stems inhibited α-glucosidase better than acarbose. The methanol extract of *P. persica* leaves and the ethyl acetate and methanol extract of *P. heterochroma* roots possess similar inhibitory activity against α-glucosidase to acarbose.

Generally, all these extracts exhibited moderate to high concentration-dependent response in α-amylase and α-glucosidase inhibitory assays.

4. Discussion

This study indicates that among the 11 plants studied, the extracts of *H. persicum*, *S. excelsa*, *P. boissieriana*, *P. persica* and *P. heterochroma* showed comparable activities against α-amylase and α-glucosidase to acarbose. In addition, *C. persicus*, *P. boissieriana*, and *P. heterochroma* showed significant antioxidant activities. Therefore, these four plants can be recommended as good natural sources for further investigations of antidiabetic drugs and antioxidants.

Table 3 – Concentrations that cause 50% inhibition (IC_{50}) values of high-effective extracts in DPPH radical scavenging, and α-amylase and α-glucosidase inhibitory assays.

Plant species	Plant part used	Extract	DPPH scavenging activity (IC_{50} μg/mL)	α-Amylase inhibition (IC_{50} μg/mL)	α-Glucosidase inhibition (IC_{50} μg/mL)
Alium paradoxum	Bulb	EA.	61.3 ± 0.9	> 238.1	> 20.0
Convolvulus persicus	Aerial parts	Met.	94.9 ± 1.4	> 238.1	> 20.0
	Root	EA.	52.7 ± 0.7	> 238.1	> 20.0
		Met.	38.9 ± 1.6	> 238.1	> 20.0
Heracleum persicum	Aerial parts	Hex.	> 200.0	41.7 ± 3.4*	5.2 ± 0.5*
		EA.	119.4 ± 6.2	> 238.1	> 20.0
	Root	Hex.	> 200.0	59.3 ± 2.9*	2.9 ± 0.1*
Pimpinella affinis	Leaf	Hex.	> 200.0	114.7 ± 8.9	12.9 ± 0.2
		Met.	74.9 ± 1.9	> 238.1	> 20.0
	Root	EA.	> 200.0	104.5 ± 6.7	> 20.0
Parrotia persica	Leaf	EA.	66.0 ± 2.6	> 238.1	8.4 ± 0.8
		Met.	57.1 ± 3.0	> 238.1	6.9 ± 0.5
Primula heterochroma	Leaf	Met.	41.7 ± 1.4	> 238.1	8.1 ± 0.4
	Root	EA.	37.9 ± 1.3	> 238.1	5.9 ± 0.7
		Met.	30.1 ± 2.8	> 238.1	6.7 ± 0.1
Pyrus boissieriana	Leaf	EA.	92.1 ± 3.1	> 238.1	> 20.0
		Met.	47.2 ± 4.0	> 238.1	4.7 ± 0.8*
	Stem	Hex.	> 200.0	> 238.1	3.2 ± 0.9*
		EA.	47.8 ± 3.5	> 238.1	2.3 ± 0.3*
		Met.	39.3 ± 1.3	186.9 ± 4.5	2.5 ± 0.6*
Smilax excelsa	Leaf	Hex.	> 200.0	99.3 ± 3.3	18.6 ± 1.9
		EA.	> 200.0	143.7 ± 5.8	> 20.0
		Met.	> 200.0	89.4 ± 3.9	> 20.0
	Stem	EA.	119.6 ± 0.1	73.9 ± 3.4*	3.9 ± 0.4*
BHT	—	—	16.7 ± 0.2	—	—
Acarbose	—	—	—	75.7 ± 2.4	6.1 ± 0.3

*IC_{50} values lower than the standard positive control.
BHT = butylated hydroxytoluene; DPPH = 2,2-diphenyl-1-picrylhydrazyl; EA = ethyl acetate; Hex. = *n*-hexane; Met. = methanol.

P. boissieriana and *P. persica* are wild trees in Hyrcania region. Native people do not use *P. boissieriana* as a medicinal plant, however, its antioxidant activity has been reported [24]. *P. persica* has been used as food coloring, food flavoring, and antifever [39,40]. Also, its antibacterial and antioxidant activities were measured. Easy access to these two plants marks them as good sources of antidiabetic natural products. *P. heterochroma* is a decorating plant in the northern part of Iran. Local people use leaves of *S. excelsa* as a food flavoring and some studies report its antioxidant and antimicrobial activities [42,43]. Now it can be regarded as a candidate for the control of diabetes mellitus. In the northern part of Iran, native people widely use leaves of *P. affinis* as a flavoring agent. Also, *H. persicum* is one of the most important plants in Iranian traditional medicine that has been used as an antiepilepic, carminative, antimicrobial, and pain killer plant [30–35]. A few studies have reported biological activities of *H. persicum*, such as antifungal, antimicrobial, and antioxidant [31,34,36,37]. Therefore, this paper can be a guideline for researchers in the field of pharmacology to make more investigations about these plants from other points of view. Also, the results can be useful for nutrition scientists.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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