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Enzyme inhibitory and radical scavenging effects of some antidiabetic plants of Turkey

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ABSTRACT

Objective(s): Ethnopharmacological field surveys demonstrated that many plants, such as *Gentiana olivieri*, *Helichrysum graveolens*, *Helichrysum plicatum* ssp. *plicatum*, *Juniperus oxycedrus* ssp. *oxycedrus*, *Juniperus communis* var. *saxatilis*, *Viscum album* (ssp. *album,* ssp. *austriacum*), are used as traditional medicine for diabetes in different regions of Anatolia. The present study was designed to evaluate the *in vitro* antidiabetic effects of some selected plants, tested in animal models recently.

Materials and Methods: α-glucosidase and α-amylase enzyme inhibitory effects of the plant extracts were investigated and Acarbose was used as a reference drug. Additionally, radical scavenging capacities were determined using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS radical cation scavenging assay and total phenolic content of the extracts were evaluated using Folin Ciocalteu method.

Results: *H. graveolens* ethanol extract exhibited the highest inhibitory activity (55.7 % ± 2.2) on α-amylase enzyme. Additionally, *J. oxycedrus* hydro-alcoholic leaf extract had potent α-amylase inhibitory effect, while the hydro-alcoholic extract of *J. communis* fruit showed the highest α-glucosidase inhibitory activity (IC_{50}: 4.4 µg/ml).

Conclusion: Results indicated that, antidiabetic effect of hydro-alcoholic extracts of *H. graveolens capitulums, J. communis* fruit and *J. oxycedrus* leaf might arise from inhibition of digestive enzymes.

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Introduction

Diabetes mellitus is a growing health problem worldwide causing severe and costly complications including blindness, cardiac and kidney diseases (1). According to Shaw *et al* (2010), the world prevalence of diabetes among adults will increase to 7.7%, and affect 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in number of adults with diabetes in developing countries and a 20% increase in developed countries (2).

Approaches to the control of blood glucose and prevention of hyperglycemia are central to the treatment of diabetes mellitus. Appetite suppressants, inhibitors of digestion, insulin secretagogues, insulin potentiators, insulin mimetics, stimulants of glucose utilization, inhibitors of gluconeogenesis and glucogenolysis are used to balance blood glucose. At present, none of these therapies either alone or in combination can redraw normal blood glucose homeostasis. Additionally many limitations exist in the use of anti-diabetic drugs; medicines available for management of diabetes exert serious side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea, and hypoglycemia. Also after prolonged treatment, drug resistance is reported for these medicines (3–6). Therefore, researchers have targeted towards the discovery of drug candidates from potential sources. Traditional medicines play an important role as starting material for drug discovery. For documentation of ethnopharmacological knowledge, many comprehensive field surveys have been conducted all over the world for years and many plants used against diabetes have been recorded (7–10).

Antidiabetic activities of plants used against diabetes in Turkey as folk medicine were studied in detail by our research group. In our research on *in vivo* antidiabetic activity of traditional medicines from 2000, seven plant species including *Gentiana olivieri* Griseb (Gentianaceae), *Helichrysum graveolens* (Bieb.) Sweet (Asteraceae), *H. plicatum* ssp. *plicatum* DC. (Asteraceae), *Juniperus oxycedrus* ssp. *oxycedrus* L. (Cupressaceae), *J. communis* var. *saxatilis* Pall. (Cupressaceae), *Viscum album* L. (ssp. *album* and ssp. *austriacum*)] (Loranthaceae) were evaluated for their antidiabetic activity (11–16). Due to their promising antidiabetic effect in *in vivo* studies, they were selected as the subject of this study.

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Table 1. General information about plants used in the study

Plant	Place of collection	Voucher No.	Part used	Extraction method	Extract	Yield % (w/w)
Gentiana olivieri Griseb.	Oğuzeli, Gaziantep	GUE 2621	Aerial part	Decoction	HA	38.7
Helichrysum graveolens (Bieb.) Sweet	Ilgaz Mt., Kastamonu	GUE 2356	Capitulum	Maceration	HA	11.5
				Infusion	Aq	10.0
H. plicatum ssp. *plicatum* DC.	Palandöken Mt., Erzurum	GUE 2355	Capitulum	Maceration	HA	19.3
				Infusion	Aq	17.5
Juniperus communis var. *saxatilis* Pall.	Akdağmadeni, Yozgat	GUE 2617	Fruit	Maceration	HA	36.0
			Leaf	Maceration	HA	29.0
J. oxycedrus ssp. *oxycedrus* L.	Akdağmadeni, Yozgat	GUE 2616	Fruit	Maceration	HA	33.3
				Infusion	Aq	26.0
			Leaf	Maceration	HA	35.2
				Infusion	Aq	17.0
Viscum album ssp. *album* L.	Bağlum, Ankara	AEF 18953	Aerial part	Maceration	HA	43.2
				Infusion	Aq	25.9
V. album ssp. *austriacum* (Wiesb.)	Kızılcahamam, Ankara	AEF 18939	Aerial part	Maceration	HA	41.2
				Infusion	Aq	27.6

AEF: Herbarium of Faculty of Pharmacy at Ankara University, GUE: Herbarium of Faculty of Pharmacy at Gazi University, HA: Hydro-alcoholic, Aq: Aqueous

The plants used in this study are well known and widely consumed as food and medicine in different regions of Anatolia. Aerial parts of *G. olivieri* are used as bitter tonic, appetizer, antidiabetic, antipyretic, stomachic, and for mental disorders. Gentians are also used in small amounts as food and beverage flavoring, in antismoking products and even as a substitute for hops in beer making. *Helichrysum* species have been used as diuretics, lithagogues, anti-asthmatics, for stomachache, and against kidney stones. The capitulums of *Helichrysum* species are used to decrease blood glucose levels and aerial parts are also marketed as herbal tea in herbal stores. Juniper berries and leaves are used for antidiabetic, diuretic, antiseptic, carminative, stomachic, antirheumatic, antifungal, and disinfectant properties in many folk medicines (13, 14). Also, berries are used as spice in European cuisine to impart a sharp, clear flavor to meat dishes, pork, cabbage, and sauerkraut dishes (18). Twigs and leaves of *V. album* (European mistletoe) are used for many therapeutic applications such as diabetes mellitus, chronic cramps, stroke, stomach problems, heart palpitations, hypertension, and breathing difficulties (15). Additionally leaves of *V. album* are used as tea for bracing and fruits are eaten fresh and pickled in Turkey (19).

The goal of the present study is to determine the inhibitory effects of the selected plants that were found to have *in vivo* antidiabetic activity on carbohydrate digestion enzymes such as *α*-amylase and *α*-glucosidase. Inhibition of these enzymes, involved in the digestion of carbohydrates, can significantly reduce the post-prandial increase of blood glucose. So, plants with inhibitory effects on these enzymes might be beneficial in diabetic patients. Oxidative stress, is one of the major problems observed during hyperglycemia and it contributes to severe complications in diabetics (20). Plants with both antidiabetic and antioxidant effects could be useful for people suffering from diabetes mellitus. Therefore, ABTS radical scavenging activity and total phenolic contents of the extracts were also determined.

Materials and Methods

Plant materials

Plant materials were collected from different localities of Turkey and identified by researchers. Voucher specimens are preserved in the herbariums of Gazi and Ankara Universities, Faculty of Pharmacy, (Ankara), Turkey. Plant names, parts used, collection sites and herbarium numbers of the plants are given in Table 1.

Preparation of extracts

Aqueous and hydro-alcoholic extracts of the plants were prepared according to folkloric usage as described in the previous *in vivo* antidiabetic activity studies (11–16). For decoctions, 1 g of air-dried plant material was added to 100 ml of distilled water and boiled on slow heat for 30 min. Infusions were prepared by pouring 100 ml of boiling water onto 1 g of dried plant material. The extraction continued for 30 min while cooling. Hydro-alcoholic extracts were prepared by maceration of 1 g of powdered material with 100 ml of ethanol (80%) at room temperature for 8 hr. Extracts were then filtered through filter paper and condensed by a rotary evaporator. Extraction yields were calculated after freeze-drying till dryness. Extract yields and other information are given in Table 1.

Assay for α-amylase inhibitory activity

The α-amylase inhibition method was performed using the chromogenic method of Ali *et al* (21). Porcine pancreatic *α*-amylase (EC 3.2.1.1, type VI, Sigma) was dissolved in ice-cold distilled water (4 U/ml). As substrate solution, potato starch (0.5 %, w/v) in 20 mM phosphate buffer (pH 6.9) was used. Experiments were carried out with three replicates.

40 µl of plant extract in DMSO, 160 µl of distilled water and 400 µl of starch were mixed in an Eppendorf tube. The reaction was initiated by the

Table 2. α-Amylase inhibitory activity of plant extracts

Material/Plant	Part used	Extract	Inh. % ± SD (µg/ml)			
			3000	1000	300	100
Acarbose	-	-	73.7 ± 0.6	67.2 ± 0.6	51.8 ± 2.9	32.6 ± 0.3
Gentiana olivieri	Aerial part	HA	39.6 ± 0.4	13.9 ± 0.6	-	-
Helichrysum graveolens	Capitulum	Aq	3.5 ± 1.8	-	-	-
		HA	55.7 ± 2.2	15.7 ± 1.6	-	-
Helichrysum plicatum	Capitulum	Aq	12.7 ± 2.8	13.7 ± 1.5	16.7 ± 1.4	17.5 ± 0.8
		HA	5.4 ± 2.3	-	-	-
Juniperus communis var. *saxatilis*	Leaf	HA	53.6 ± 0.8	2.4 ± 2.4	-	-
	Fruit	HA	29.8 ± 1.2	22.6 ± 1.7	-	-
J. oxycedrus ssp. *oxycedrus*	Fruit	Aq	8.2 ± 6.5	-	-	-
		HA	52.6 ± 0.8	39.0 ± 1.0	-	-
	Leaf	Aq	42.1 ± 2.0	11.3 ± 4.6	-	
		HA	51.7 ± 0.9	25.6 ± 0.9	25.2 ± 1.3	25.0 ± 0.7
Viscum album ssp. *album*	Aerial part	Aq	14.0 ± 4.2	2.2 ± 1.5	-	-
		HA	8.7 ± 2.3	2.4 ± 1.2	2.0 ± 1.8	1.8 ± 0.6
V. album ssp. *austriacum*	Aerial part	Aq	-	-	-	9.0 ± 3.3
		HA	44.3 ± 4.1	10.8 ± 3.0	2.6 ± 2.1	-

n=3, SD: Standard deviation, :no activity, HA: Hydro-alcoholic, Aq: Aqueous

addition of 200 µl of the enzyme solution. The tubes were incubated at 37˚C for 5 min. After that, 200 µl of this mixture was added into another tube containing 100 µl DNS color reagent solution (96 mM 3, 5-dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) and put into a 85˚C heater. After 15 min, this mixture was diluted with 900 µl distilled water and taken from the heater. Tubes were cooled on ice and the absorbance of the mixture was read at 540 nm. Acarbose was used as the positive control. The absorbance (A) due to maltose generated was calculated according to following formula:

A_{540nm} control or plant extract=A_{540nm} Test−A_{540nm} Blank

The amount of maltose generated was calculated by using the maltose standard calibration curve (0 – 0.1% w/v) and the obtained net absorbance. Percent of inhibition was calculated as:
% inhibition = [1-(mean maltose in sample/mean maltose in control)] × 100

Assay for α-glucosidase inhibitory activity

α-Glucosidase activity was performed according to the method of Lam *et al* (22). α-Glucosidase type IV enzyme (Sigma Co., St. Louis, USA) from *B. stearothermophilus* was dissolved in 0.5 M phosphate buffer (pH 6.5) (3 U/ml). The enzyme solution (20 µl) and test extracts (10 µl) dissolved in MeOH-H_2O (1:9, v/v) were preincubated in a 96-well microtiter plate for 15 min at 37˚C. After that, the substrate solution [10 µl, 20 mM *p*-nitrophenyl-α-d-glucopyranoside (NPG), Sigma] in the same buffer was added. The mixture was incubated for 35 min at 37˚C. The increase in the absorption at 405 nm due to the hydrolysis of NPG by α-glucosidase was measured by an ELISA microtiter plate reader. Acarbose (Bayer Group, Turkey) was used as a positive control. The inhibition percentage (%) was calculated by the equation:

Inhibition (%) = [1 − (A_{sample}/$A_{control}$)] × 100

IC_{50} calculations were done by using Sigma Plot 12.0 software. Minimum of eight different concentrations prepared from the stock solutions of extracts were used for calculating the IC_{50} value. The logarithmic concentrations (10.000–0.1 µg/ml) were chosen.

Assay for scavenging activity of ABTS radical cation

ABTS radical cation ($ABTS^{\cdot+}$) scavenging assay was achieved by using the spectrophotometric methods of Re *et al* (23) and Meot-Duros *et al* (24) with slight modifications. ABTS (7 mM) was dissolved in distilled water and the ABTS radical cation was generated by adding 2.45 mM potassium per-sulfate. The radical production was completed after incubation for 16 hr in the dark at 20˚C. Absorbance of ABTS solution was adjusted to 0.7 ± 0.02 at 734 nm by the addition of phosphate buffer solution (PBS) at pH 7.4. 1 ml diluted ABTS solution was added to 10 µl of extract (PBS or Trolox). Samples were vortexed and their absorbances were read *versus* PBS blank at 734 nm. Trolox was used as the positive control. The inhibition percentage was calculated according to the following formula:

Inhibition percentage= [1-($A_{extract}$ /$A_{control}$)]x100

Determination of total phenol content

The extracts (100 µl) were mixed with 0.2 ml Folin-Ciocalteu reagent, 2 ml of H_2O, and 1 ml of 15 % Na_2CO_3, respectively. The absorbance of mixture was measured at 765 nm after 2 hr at room temperature. The mean of three readings was used and the total phenol content was expressed in mg of gallic acid equivalents (GAE)/g extracts (25). The coefficient of determination was r^2= 0.9957.

Table 3. α-Glucosidase inhibitory activity of plant extracts and total phenol content (TPC)

Material/Plant	Part used	Extract	IC_{50} (mg/ml)	TPC ± SD
Acarbose	-	-	0.0009	NT
Gentiana olivieri	Aerial part	HA	0.1982	57.4 ± 2.7
Helichrysum graveolens	Capitulum	HA	0.7129	143.4 ± 9.4
		Aq	2.1979	92.9 ± 2.0
H. plicatum ssp. *plicatum*	Capitulum	HA	0.8570	139.5 ± 6.5
		Aq	5.0933	85.6 ± 15.7
Juniperus communis var. *saxatilis*	Fruit	HA	0.0044	21.0 ± 10.1
	Leaf	HA	0.0843	212.1 ± 9.9
J. oxycedrus ssp. *oxycedrus*	Fruit	HA	-	4.8 ± 2.2
		Aq	0.8054	24.8 ± 0.7
	Leaf	HA	0.0473	191.0 ± 1.3
		Aq	0.2606	160.4 ± 2.7
Viscum album ssp. *album*	Aerial part	HA	0.7962	21.2 ± 2.0
		Aq	3.7411	32.0 ± 0.2
V. album ssp. *austriacum*	Aerial part	HA	0.6653	35.8 ± 1.3
		Aq	1.3583	47.9 ± 0.8

Total phenol content data is expressed in mg equivalent of gallic acid (GAE) to 1 g of extract.
SD: Standard deviation, NT: Not tested, -:no activity, HA: Hydro-alcoholic, Aq: Aqueous

Statistical analysis

All analyses were carried out in triplicates and the results were averaged. All values are expressed as the mean±standard deviation (SD); linear regression analyses and IC_{50} calculations were done by using SigmaPlot 12.0 software. Microsoft Excel software was used to calculate correlation coefficients to determine the relationship between 2 variables.

Results

α-amylase inhibitory activity

α-Amylase inhibitory activities of the plant extracts were evaluated at 4 different logarithmic doses (3000, 1000, 300, 100 µg/ml) and results were given in Table 2. All extracts except *H. plicatum* aqueous extract, showed a dose dependent inhibitory effect on α-amylase enzyme. All the extracts exerted inhibitory activity at tested doses in varying proportions (3.5 – 55.7 % at 3000 µg/ml). *H. graveolens* hydro-alcoholic extract exhibited the highest inhibitory activity at 3000 µg/ml (55.7 %), while the inhibition percentage of the reference drug Acarbose was found to be 73.7 %. On the other hand, *J. oxycedrus* ssp. *oxycedrus* leaf hydroalcoholic extract possessed a continuous inhibitory effect on α-amylase enzyme between 100–3000 µg/ml (25.0–51.7%).

α-glucosidase inhibitory activity

α-Glucosidase inhibitory activities of the plant extracts were evaluated at 5 different logarithmic doses between 0.3–10000 µg/ml; the calculated IC_{50} values are given in Table 3. All the extracts showed dose dependent inhibitory effect on α-glucosidase enzyme. *J. communis* var. *saxatilis* fruit hydro-alcoholic extract possessed the highest inhibitory effect and its IC_{50} value was found to be the lowest (IC_{50} = 0.0044 mg/ml) among all extracts. IC_{50} value of reference drug (Acarbose) was 0.0009 mg/ml. *H. plicatum* ssp. *plicatum* capitulum aqueous extract (IC_{50} = 5.0933 mg/ml) and *V. album* ssp. *album* aerial part aqueous extract (IC_{50} = 3.7411 mg/ml) exerted the lowest enzyme inhibitory activity.

ABTS radical cation scavenging activity

ABTS radical cation decolorization assay is a useful method for determining the antioxidant capacity of hydrogen donating antioxidants. $ABTS^{\cdot+}$ is a blue chromophore produced by the reaction between ABTS salt and potassium per-sulfate (26). After addition of extracts to ABTS radical cation, a strong reduction was observed and the blue color turned to white immediately in some extracts (*H. plicatum* capitulum hydro-alcoholic, *J. communis* var. *saxatilis* leaf hydro-alcoholic, *J. oxycedrus* ssp. *oxycedrus* leaf aqueous and hydro-alcoholic extracts) at 3000 µg/ml concentration. Trolox used as a positive control, showed ABTS radical cation scavenging activity at all tested concentrations (100–3000 µg/ml). *J. oxycedrus* ssp. *oxycedrus* fruit aqueous extract exerted the lowest radical scavenging activity (0–7.5%). The results of ABTS radical cation decolorization assay is given in Table 4.

Total phenol content

Total phenol contents of all the extracts were measured and the results were shown in Table 3. The highest total phenol content was found in *J. communis* var. *saxatilis* leaf hydro-alcoholic extract (212.1 ± 9.9 mg GAE/1 g extract) while the lowest was found in *J. oxycedrus* ssp. *oxycedrus* fruit hydro-alcoholic extract (4.8 ± 2.2 mg GAE/1 g extract). Results presented in Table 3 show that there is a positive correlation between total phenol contents and ABTS radical scavenging activity of plant extracts (correlation coefficient= r = 0.8875 at 3000 µg/ml). However, the extracts with potent antioxidant activity and rich in phenolics did not show high inhibition on digestion enzymes. No correlation was observed between total phenol content and α-amylase/α-glucosidase inhibitory activity (r = 0.3959 and r = 0.1669 at 3000 µg/ml respectively). Additionally correlation between radical

Table 4. ABTS radical scavenging activities of plant extracts

Material/Plant	Part used	Extract	Inh. % ± SD (μg/ml)			
			3000	1000	300	100
Trolox	-	-	>100	99.5 ± 0.25	38.9 ± 1.12	7.5 ± 0.72
Gentiana olivieri	Aerial part	HA	54.3 ± 1.20	20.5 ± 2.41	7.6 ± 1.03	2.6 ± 0.29
Helichrysum graveolens	Capitulum	Aq	77.0 ± 0.7	36.5 ± 0.74	11.2 ± 0.7	4.3 ± 0.91
		HA	88.5 ± 1.89	42.6 ± 1.95	15.0 ± 0.40	6.5 ± 2.53
H. plicatum	Capitulum	Aq	75.7 ± 0.61	36.1 ± 0.64	16.3 ± 0.8	2.7 ± 0.36
		HA	98.4 ± 2.66	53.5 ± 0.67	19.5 ± 0.36	6.8 ± 0.79
Juniperus communis var. *saxatilis*	Leaf	HA	99.5 ± 0.35	68.9 ± 1.03	24.3 ± 6.37	-
	Fruit	HA	42.5 ± 1.2	12.2 ± 0.57	-	-
J. oxycedrus ssp. *oxycedrus*	Fruit	Aq	7.5 ± 0.96	-	-	-
		HA	48.9 ± 0.55	19.4 ± 1.81	5.1 ± 0.70	2.3 ± 2.77
	Leaf	Aq	97.8 ± 0.83	37.2 ± 0.89	5.6 ± 0.5	-
		HA	97.8 ± 0.25	46.0 ± 0.51	4.7 ± 1.40	-
Viscum album ssp. *album*	Aerial part	Aq	33.7 ± 1.19	12.9 ± 2.05	-	-
		HA	50.2 ± 1.64	21.7 ± 2.78	13.3 ± 0.7	5.7 ± 1.44
V. album ssp. *austriacum*	Aerial part	Aq	47.5 ± 1.45	7.03 ± 0.06	-	-
		HA	56.6 ± 0.45	16.7 ± 0.7	8.9 ± 1.1	-

n=3, SD: standard deviation, -: no activity, HA:Hydro-alcoholic, Aq:Aqueous

scavenging and enzyme inhibitory activities of tested plant extracts were examined. No correlation was observed between ABTS radical scavenging and *α*-amylase/*α*-glucosidase inhibitory activity (r= -0.0876 at 3000 μg/ml and r= -0.1175 respectively).

Discussion

Hyperglycemia has been a classical risk factor in the development of diabetes and its complications. Therefore, control of blood glucose levels is critical in the early treatment of diabetes mellitus. One of the important therapeutic approaches is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of the enteric enzymes including *α*-glucosidase and *α*-amylase present in the intestinal brush border (27, 28). The inhibition of these enzymes has been a strong option in the prevention of diabetes. So, inhibitors like Acarbose, voglibose, and miglitol are widely used in type 2 diabetic patients nowadays. Moreover, studies are being carried out to find new amylase and glucosidase inhibitors from natural sources (29–31).

The aim of this study is to clarify the mechanism of action of selected plants on carbohydrate metabolism. For this purpose, the inhibitory effect of 15 extracts obtained from different parts of 7 plants on *α*-glucosidase and *α*-amylase were assessed and compared with the *α*-glucosidase inhibitor, Acarbose. Also, radical scavenging activity and total phenol content of the extracts were investigated.

In our previous studies, we demonstrated significant hypoglycaemic and antidiabetic activities of hydro-alcoholic extracts of *H. graveolens* capitulums*, J. oxycedrus* ssp. *oxycedrus* leaves and *J. communis* var. *saxatilis* fruits in normoglycaemic, glucose loaded and streptozotocin-induced diabetic rats. In the present study, these extracts which were found to have potent antidiabetic activity, have also shown high inhibitory effect on enzymes that have an important role in carbohydrate metabolism. There was no correlation between total phenol content and *α*-amylase/*α*-glucosidase inhibitory activity of these plant extracts.

Many studies were conducted on the chemical profile of the selected medicinal plants. Orhan *et al* (13, 14) isolated and identified many compounds that are responsible for the antidiabetic activity of *J. oxycedrus* ssp. *oxycedrus* (Joso). Through *in vivo* bioactivity-guided fractionation processes, shikimic acid, 4-O-*β*-D-glucopyranosyl ferulic acid, and oleuropeic acid-8-O-*β*-D-glucopyranoside were isolated from the active subfractions of Joso fruit hydro-alcoholic extracts as the active components (14). Jeong *et al* (2012) showed strong inhibitory effects of ferulic acid derivatives on *α*-amylase and *α*-glucosidase enzymes (32). Therefore, we propound that *α*-amylase inhibitory effect of Joso fruit hydro-alcoholic extract might be produced by the presence of ferulic acid and other chemical constituents. Additionally, the major antidiabetic compounds in subfractions of Joso leaves were identified as fatty acids such as palmitic, linoleic, and linolenic acid (13). Su *et al* (2013) investigated the inhibitory mechanisms of fatty acids on key enzymes related to type 2 diabetes. Oleic and linoleic acids were found to have potent inhibitory effects on *α*-glucosidase activity (33). Thus, fatty acids might contribute to the *α*-glucosidase enzyme inhibitory effects of other active compounds found in the Joso leaf hydro-alcoholic extract.

Leaves and fruits of *J. communis* var. *saxatilis* contain relatively high amounts of monoterpene hydrocarbons such as *α*-pinene, limonene and *β*-myrcene (34). The main monoterpene component for these parts of the plant was *α*-pinene. Başak and Candan (2013) found that *α*-pinene in *Laurus nobilis* essential oil inhibited *α*-glucosidase (35). On the other hand, *J. communis* var. *saxatilis* leaf hydroalcoholic extract showed significantly *α*-amylase inhibitory effect which may be due to the presence of some secondary metabolites such as lignans, coumarins, sterols, aliphatic compounds, and other terpenes in the hydroalcoholic extract (34).

Results of α-amylase inhibitory activity assay showed that *H. graveolens* hydro-alcoholic extract has *in vitro* enzyme inhibition in a degree similar to Acarbose at 3000 µg/ml. Flavonoids, acetophenones, phloroglucinol, pyrones, triterpenoids, and sesquiterpenes are secondary metabolites of the genus *Helichrysum* (36). Additionally, Albayrak *et al* (2010) reported the presence of chlorogenic acid, caffeic acid, ferulic acid, syringic acid, apigenin, apigenin-7-glucoside, and hesperidin; luteolin, naringenin, quercetin, resveratrol in the methanol extracts of *H. graveolens,* and chlorogenic acid were found to be the major phenolics in the extract (37). Narita *et al* (2008) reported the strong inhibitory effect of chlorogenic acid and its derivatives on porcine pancreas α-amylase (38). It is considered that high phenolic content (143.4 mg GAE/1 g extract) of hydro-alcoholic extract of *H. graveolens* capitulums might support the enzyme inhibitory effect of other constituents like chlorogenic acid and its derivatives.

H. plicatum capitulum hydro-alcoholic, *J. communis* var. *saxatilis* leaf hydro-alcoholic, and *J. oxycedrus* ssp. *oxycedrus* leaf aqueous and hydro-alcoholic extracts have shown strong ABTS radical cation scavenging activity. Antioxidant effects of these plants might cooperate with their antidiabetic activity and these plants might be a better choice for complementary remedies for type 2 diabetic patients.

Conclusion

This is the first study on the *in vitro* antidiabetic activities of these seven plants: *G. olivieri*, *H. graveolens*, *H. plicatum* ssp. *plicatum*, *J. oxycedrus* ssp. *oxycedrus*, *J. communis* var. *saxatilis*, and *V. album* (ssp. *album* and ssp. *austriacum*). These seven plants with previously reported *in vivo* antidiabetic effect were tested for enzyme inhibitory and radical scavenging activities. Among these, *H. graveolens* hydro-alcoholic extract, *J. communis* leaf hydro-alcoholic extract and *J. oxycedrus* leaf and fruit hydro-alcoholic extracts were found to have inhibitory effect on α-amylase. On the other hand, hydralcoholic extracts of *J. communis* (leaf, fruit) *J. oxycedrus* (leaf) had potent inhibitory activity on α-glucosidase. In conclusion, the findings of this investigation indicate that these plants might be ameliorate hyperglycemia in type 2 diabetics by their inhibitory effect on α-glucosidase and α-amylase. It is concluded that further studies are needed to explain the mechanism of actions of the other extracts and their active constituents.

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