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Original article

Gundelia Tournefortii L. (Kenger): Determination of in vitro Antidiabetic Activities

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Abstract. Diabetes mellitus is a chronic disease that negatively effects daily life. Synthetic drugs, which are inhibitors of α-glucosidase and α-amylase enzymes, are used in its treatment. These drugs may have adverse reaction on organs such as the liver. Therefore, it is important to include plants that inhibit these enzymes in the diet in the treatment of Diabetes mellitus. *Gundelia tournefortii L.* (Kenger) is an edible wild natural plant that is widely used as traditional food. In this study, biochemical characterization of the *Gundelia tournefortii L.* (Kenger) extracts was performed. The inhibition effect of the extract on α-amylase and α-glycosidase enzymes was also investigated. Total phenolic content, antioxidant activity and chemical composition of *Gundelia tourneforti L.* extracts were determined. Chemical composition of extracts was determined by using GC/MS technique. IC_{50} values of α-amylase and α-glucosidase enzymes of the *Gundelia tournefortii L.* ethanolic extracts of stalk were determined as 4.18 ± 0.08 mg/mL and 9.77±1.2 mg/mL, respectively. The results showed that *Gundelia tournefortii L.* could be used as a supplementary food in the treatment of Type 2 diabetes.

Key words: Gundelia tournefortii L, Diabetes mellitus, α-amylase, α-glucosidase, phenolic, DPPH, FRAP

Introduction

Diabetes mellitus is a chronic disease with increased risk of lipid and lipoprotein metabolism, insulin resistance and cardiovascular, which causes damage in various organs and systems (1). Hyperglycemia in diabetic patients increases the formation of free radicals. Oxidative stress causes the balance between free radicals and antioxidants to deteriorate in favor of free radicals (2). Patients go through a difficult process regarding diabetes complications and transformation of lifestyle changes into behavior. In this context, patients can often turn to complementary therapies in addition to medical treatments. Natural products (especially herbs) are known to be important therapeutics in chronic diabetes, as they lower blood sugar and hyperlipidemia (3).

There are many compounds, both primary and secondary, in plants. They also contain complex combinations of many secondary compounds that act synergistically to elicit their beneficial effects, and the medicinal effects of herbs can be very different as these combinations are different for each plant variety (3-4). Secondary compounds can be classified as phenolic compounds (such as phenylpropanoids, flavonoids, catechins, tannins), terpenoids (such as mono and sesquiterpenes, saponins, iridoids) and polysaccharides. All these primary and secondary compounds can cause

some medical effects in humans. Examples include antimicrobial effect, antidiabetic effect, antidepressant effect, muscle relaxant and sedative effect (5-6).

Gundelia tournefortii L., a specific medicinal plant, grows in temperate regions of Asia, Egypt, Iran, Israel, Jordan, Turkey, Azerbaijan and Turkmenistan (7). Known by different names such as kenger, flint, thorn, the flowers bloom in May. Kenger grass is veined, whitish hairy, and stem leaves are sessile. It has red to purple flowers. Its flowers, leaves, seeds and stalks are widely used as a food source from past to present.

The aim of this study is to investigate antidiabetic activity of *Gundelia tournefortii* L. grown in Turkey's mountainous regions. For this purposes ethanolic and aqueous extracts from different parts of the plant were prepared. The inhibitory effect of these extracts on α-amylase and α-glucosidase enzymes was determined. These two enzymes have key role in carbohydrate metabolism and they are the first target in the treatment of Type 2diabetes mellitus.

Materials and Methods

Preparation of Plant Extract

Gundelia tournefortii L. was freshly collected in 2019. The roots and stalks of plant are air dried. 10 g of both parts of the plant were weighed and grounded and extracted separately with ethanol at room conditions for 24 hours and then sonicated for 2 hours (ultrasonic Elma Schmidbauer GmbH, Germany). Then the mixtures were filtered and evaporated. (IKA-Werke, Staufen, Germany). The residue was dissolved in minimum volume of ethanol and stored at 4 ° C. The same procedures were repeated for the aqueous extracts.

Determination of Total Phenolic Content

Total phenolic content of the extracts was determined using the Folin–Ciocalteu method (8-9). Calibration curve was obtained by using gallic acid (GA) standard, the results were expressed as mg GAE/100g.

Determination of Total Flavanoid Content

The total flavonoid content was determined according to Fukumoto and Mazza (2000). Quercetin (QE) was used as a standard. Results were expressed as mg QE/100g (10).

Determination of the Chemical Composition of the Extracts

The chemical composition of *Gundelia tournefortii L.* extracts was determined using GC/MS technique. For this purpose, Agilent 7890A GC system HP5-MS capillary column (30 m * 0.25 mm * 0.5 mm) were used. The relative proportions of the components were determined using the Wiley library (6).

Determination of Ferric Reducing Power Antioxidant Activity

The FRAP method is the most commonly used method for determining the antioxidant capacity of natural products, and it is a method based on the reduction of iron (III) ion in the Fe(III)-TPTZ complex by antioxidants (11). Fe(III), which is reduced by the antioxidant substances in the solution, gives absorbtion at 593 nm. Results for FRAP activity were expressed as mM Fe^{+2}/mL.

DPPH Radical Scavenging Activity

DPPH • radical (2,2-diphenyl-1-picrylhydrazyl) is a commercially available radical. 100 μM methanolic solution of this purchased radical was used in the trials. The extracts of the samples were diluted with their own solvents and prepared in different concentrations. Equal volume (750 μL) of DPPH • solution and sample solutions were mixed and left at room temperature for 50 minutes. At the end of this period, absorbances were read at 517 nm, at which DPPH gives maximum absorbance. By drawing the concentration graph against the absorbances read, IC_{50} values are calculated and expressed as mg/mL (12).

Enzyme Inhibition Studies

α- *Amylase Enzyme Inhibition*

While determining the enzyme activity, equal volume of enzyme solution was added onto 300 µL 1% of starch as substrate. This mixture was incubated at the 37 °C for 30 minutes. At the end of this period, an equal volume of DNS solution was added. Then, the tubes were placed in a boiling water bath for 5-10 min for color production. Later, tubes were cooled in an ice bath. After the temperature of the tubes reached the room temperature, their absorbance against blank was measured at 550 nm. Blank tube contained distilled water instead of enzyme solution. For the inhibition study, extracts in five different concentrations were added into the reaction medium and enzyme activity was calculated and dose response curve was generated for IC_{50} determination. Acarbose was used as reference inhibitor (13).

α- *Glucosidase Enzyme Inhibition*

α-glucosidase activity was performed according to the method described by Gholamhoseinian (14). Enzyme reaction was carried out in 0.1 M pH 6.8 phosphate buffer using *p*-nitrophenyl-

α-D-glucopyranoside as substrate. 5 µL of substrate, enzyme solution (0.1 U) and 900 µL of phosphate buffer (50mM) were mixed. The mixture was incubated at 37 °C and the absorbance values at 405 nm were recorded (14). For the inhibition study, extracts in five different concentrations were added into the reaction medium and enzyme activity was calculated and dose response curve was generated for IC_{50} determination.

Results

In this study, ethanolic and water extracts were prepared from *Gundelia tournefortii L.* and biochemical characterization of the prepared extracts were performed. Than, the inhibition effect of the plant extracts on α-amylase and α-glucosidase enzymes, which are important in the treatment of Diabetus mellitus, were determined (Table 1). Chemical characterization of plant extracts were performed using GC/MS and the results were presented in Table 2.

Discussion

Studies show the relationship of diabetes and diabetes complications with reactive oxygen species emphasize that nonenzymatic glycation, metabolic stress caused by changes in energy metabolism, sorbitol pathway activity, hypoxia and tissue damage caused by ischemia-reperfusion increase free radical production and change the antioxidant defense system (15). It has

Table 1. Biochemical and enzyme inhibition properties of *Gundeliatournefortii L.*extract

	Total Phenolic Content mg GAE/100g	Total Flavonoid Content mg qe/100 g	Ferric reducing Activity (FRAP) mM Fe^{+2}/mL	DPPH IC_{50} mg/mL	α- Amylase IC_{50} mg/mL	α- Glucosidase IC_{50} mg/mL
Stalk (EtOH)	145.6±0.9	22.3±0.2	0.58±0.14	1.55±0.05	4.18±0.8	9.77±1.2
Roots (EtOH)	194.3±1.2	50.8±0.3	0.96±0.12	1.43±0.06	3.35±0.8	9.18±1.3
Stalk (Water)	102.4±1.1	14.2±0.1	0.33±0.12	1.63±0.06	5.17±0.9	10.18±1.2
Roots (Water)	115.2±0.7	9.8±0.1	0.37±0.12	1.60±0.06	4.76±1.0	10.13±1.4
Acarbose					3.21±0.7	3.56±0.8

Table 2. Chemical composition of *Gundeliatournefortii L.*

Compounds	Stalk (EtOH)		Roots (EtOH)	
	RT	Area (%)	RT	Area (%)
Octane	3.487	0.15	3.486	0.26
α-pinene	6.103	0.09	6.103	0.18
Decane	6.821	0.42	6.820	0.27
Dodecane	10.042	1.63	10.044	2.16
Hexadecane	11.766	0.20	11.765	0.43
Benzene, 4-ethyl-1,2- dimethyl	13.383	1.83	13.384	2.51
Pentadecane	14.782	0.36	14.782	0.27
α-Farnesene	16.696	0.40	16.695	0.58
Lauric acid	17.671	12.20	17.672	17.52
Linalool	19.353	0.65	19.352	0.73
Undecane, 4-cyclohexyl	19.432	2.02	19.432	2.28
Oleic acid	19.766	0.23	19.767	0.51
Caryophyllene oxide	19.931	1.28	19.930	1.04
Myristic acid	20.300	3.86	20.301	5.40
1-Hexadecanol, 2-methyl	20.760	3.77	20.762	3.94
Hexahydro farnesyl acetone	21.229	1.68	21.227	2.47
Pentadecanoic acid	21.485	2.51	21.485	3.08
Palmitic acid	23.633	14.11	23.632	17.26
Octadecane	27.257	2.57	27.258	3.11
Eicosane	29.598	0.15	29.598	0.38
Phenol, 2,4-bis(1- phenylethyl)	31.220	1.34	31.221	2.67
Heneicosane	33.379	2.18	33.378	3.45

been suggested that free radical formation increases and radical binding systems decrease in diabetes and it has been argued that diabetics may need more antioxidants (16). Oxidative stress plays an important role in the pathogenesis of diabetes and subsequent complications in diabetics (17). Increased DNA, protein and lipid peroxidation products are indicators of high oxidative stress in diabetics. The etiology of oxidative stress in diabetes is caused by many different mechanisms. For example, with the autoxidation of glucose, glycosylated proteins and antioxidant enzymes cannot detoxify oxygen radicals, increasing oxygen radical production. In addition to these mechanisms, there are two other mechanisms responsible for the production of oxygen radicals in diabetes. The first is that high glucose levels stimulate cytochrome P450-like activity through increased NADPH level as a result of glucose metabolism. The other is the ketosis which is the distinctive feature of Type 1 diabetes, increases oxygen radical production in diabetic patients (18). Pharmacological treatment of *Diabetes mellitus* is based on hypoglycemic drugs and insulin. Due to the side effects of these therapeutic agents, interest in herbal treatment methods as supportive therapy is increasing. In search of new antidiabetic drugs with less side effects, there has been a great interest in herbal sources for treatment methods. Herbal medicine treatment approach is seen as a practical and low cost approach. With this approach, determining the inhibitory effects of plants used as food in daily life on important enzymes such as α-amylase and α-glycosidase also reveals supplementary foods that can be used in diabetic patients (19).

In 2019, Dalar et al., investigated the effect of Gundelia species on several clinically important enzymes. In their study, they investigated the biopharmaceutical potency and bioactive compounds of *Gundelia rosea* seed. Antioxidant activity findings revealed that the ethanol extract contained high levels of total phenolics (55.3 mg of Gallic acid Equal/g extract) and had high reduction capacities (1683 μmol Fe2 + and 214.1 mg Trolox Equal/g extract for FRAP and CUPRAC, respectively). Free radical scavenging and total antioxidant properties were also reported. The inhibitory ability of the extracts against selected isolated enzymes revealed that the ethanol extract had pronounced inhibitory activities on amylase (0.61 mmol Acarbose Equivalent), glucosidase (11.91 mmol Acarbose Equivalent). The findings of mentioned study confirm the traditional use of Gundelia rosea and could be a candidate for a new biopharmaceutical agent for public health issues (20). Çoruh et al., (2007) determined antioxidant activities of both the above ground parts and seeds of Gundelia tournefortii; They investigated antioxidant activity by using both 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation inhibition method. The seeds were found to have higher antioxidant potential than the above-ground parts, with IC_{50} values of 0.073 mg/mL for DPPH scavenging. In addition, the total phenolic contents of Gundelia tournefortii L. extracts, particularly seed extracts, correlate with high antioxidant activity, with 105.1 ± 8.7 μg gallic acid equivalent (GAEs) per mg seed extract (21).

Farhang et al., 2007, in their study, identified the chemical components of Gundelia Tournefortii L. in some habitats in the Central Zagros region of Iran. For this purpose, Gundelia tournefortii L. was collected from some of its natural habitats in the region and air dried. The chemical composition of the plant was illuminated using GC / MS. Accordingly, the main components were reported as palmitic acid (12.48%), lauric acid (10.59%), alpha ionene (6.68%), myristic acid (4.45%), 1-hexadecanol, 2-methyl (3.61%), phytol (3.6%) and beta turmeron (3.4%) (6). Kadan et al., 2018, in their in vitro study tested the chemical composition, cytotoxicity and antidiabetic activity of two different extracts of *Gundelia Tournefortii* L. It was reported that *Gundelia tournefortii* L. contained palmitic acid, glycerol, linoleic acid (22). In another study Konak et al., (2017) determined the total phenolic content and antioxidant capacity of Gundelia tournefortii collected from the Diyarbakır region. Folin-Ciocalteu method was used to determine the total phenolic content and DPPH method was used for the determination of antioxidant capacity. The total extractable phenolic content of the stalk parts of the plant was reported to be 700.21 mg GAE / 100g. In their study, researchers stated that the plant *Gundelia tournefortii* L. was a potential source of antioxidants and could be consumed daily as a natural antioxidant source (7). The findings of present study are correlated with the literature datas.

Conclusion

Diabetes mellitus (Type 2) is a disease that can occur due to eating habits. It is possible to control this disease by eating antioxidant-rich foods regularly. In this study, biochemical characterization of *Gundelia tournefortii L.* extracts were performed. α-amylase and α-glucosidase enzyme inhibition properties were determined The data obtained show that *Gundelia tournefortii L.* could be used as a supplementary food in the treatment of *Diabetes mellitus* (Type 2).

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