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Antioxidant potential and carbohydrate digestive enzyme inhibitory effects of five *Inula* species and their major compounds

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ABSTRACT

The present study was designed to examine *in-vitro* antidiabetic activities of different extracts of flowers, leaves and roots of *Inula helenium* ssp. *turcoracemosa*, *I. montbretiana*, *I. peacockiana*, *I thapsoides* ssp. *thapsoides* and *I. viscosa* extracts. *I. viscosa* and *I. montbretiana* flower, *I. thapsoides* and *I. viscosa* leaf and *I. helenium* root methanol extracts exhibited remarkable α-glucosidase inhibitory activity. Additionally, α-amylase inhibitory activities of the extracts were moderate at only 3000 μg/mL. Based on the results of *in-vitro* antidiabetic activity tests; antioxidant activities, total phenol and flavonoid contents of the most promising extracts were evaluated. To identify compounds responsible for the antidiabetic activity, major compounds of *Inula* species were analyzed for their *in-vitro* enzyme inhibitory activity. Quercetin, luteolin and rutin exhibited a significant inhibition on α-glucosidase at 10 mM concentrations. Consequently, *Inula* species could potentially be used by diabetic patients for their antidiabetic and antioxidant activities.

1. Introduction

Diabetes mellitus is one of the most widespread metabolic disorders in all over the world. According to data published by the International Diabetes Federation in 2010, approximately 300 million people suffered from diabetes in the world (Chang et al., 2013). Chronic hyperglycemia induces the production of excessive amounts of reactive oxygen species in tissues and this progress can lead to various health problems in the kidney, heart, eye, liver and central nervous system, and this progress can cause serious tissue and organ damages. Therefore, the discovery of antidiabetic compounds or extracts with antioxidant potential is essential for treatment of diabetes mellitus. For this reason, the studies have been focused on antioxidant, *in vitro* and *in vivo* antidiabetic potentials of plants based products.

The genus *Inula* comprises more than one hundred species growing in Africa, Asia and Europe, predominantly in the Mediterranean area. The traditional uses of *Inula* species have been mentioned firstly by the Roman and Greek medical doctors. The members of the genus have widely been used in Traditional Chinese medicine as well as Ayurvedic and Tibetan medicinal systems for the treatment of various diseases such as bronchitis, diabetes, fever, hypertension and inflammation (Seca et al., 2014).

Food and Drug Administration permits the use of alcoholic beverages obtained from *I. helenium* rhizomes and roots as natural flavouring substances and natural adjuvants in foods (Food and Drug Administration, 2014) and The Council of Europe lists *I. helenium* as a natural food flavouring. In Turkey, flowers, leaves and roots of *I. heterolepis*, *I. viscosa*, *I. oculus-christi* and *I. thapsoides* subsp. *thapsoides* are consumed as food raw or cooked (Ertuğ, 2014). On the other hand, a large number of studies are being carried out on *Inula* species due to their important

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ethnomedicinal uses. Antidiabetic activity is one of the most important activities of *Inula* species (Zhang et al., 2012; Seca et al., 2014). *I. hupehensis* and *I. viscosa* are reported to be used in folk medicine to treat diabetes (Zeggwagh et al., 2006; Qin et al., 2011). Leaves and flowers of *I. viscosa* together with *I. helenium* and *I. conyza* are included in the list of medicinal plants used traditionally to treat diabetes mellitus in Morocco (Eddouks et al., 2007). Additionally, *I. viscosa* is mentioned as a plant used for the treatment of diabetes in Israel (Yaniv et al., 1987). Also, standardized extract prepared from leaves of cultivated *I. viscosa* by proprietary methods of Argo-technology has been used in pharmaceutical and cosmetic industry in Israel (Inulav, 2016). This extract having broad spectrum activity against foliar diseases of crop plants has been utilized as natural pesticide (Wang et al., 2004). Moreover, *Inula* plants such as *I. helenium* and *I. japonica*, take place in commercial herbal preparations (Zhao et al., 2006; Han et al., 2010; Seca et al., 2014). With respect to food and ethnobotanical usage of *Inula* species on diabetes, we decided to build this work concerning the *in-vitro* antidiabetic and antioxidant potentials of Turkish *Inula* plants.

Small intestinal α-glucosidase and pancreatic α-amylase are important enzymes supposed to regulate dietary carbohydrate digestion in humans. Inhibition of these enzymes may block the carbohydrate digestion and glucose absorption to suppress hyperglycemia. We used these enzymes for determining the *in-vitro* antidiabetic activity of the selected *Inula* species and authentic compounds isolated and quantified from these sources. To our best knowledge, this is the first study about inhibitory activity on carbohydrate digestive enzymes of selected *Inula* species.

The main purpose of this work is to investigate the α-glucosidase and α-amylase inhibitory activities of five *Inula* taxa together with to evaluate the antioxidant potential, total phenol and flavonoid contents of the most active enzyme inhibitor extracts. Flowers, leaves and roots of *I. helenium* ssp. *turcoracemosa*, *I. montbretiana*, *I. peacockiana*, *I. thapsoides* ssp. *thapsoides and I. viscosa* were extracted separately with water, methanol and ethyl acetate to obtain the crude extracts used in this work. After evaluation of α-glucosidase and α-amylase inhibitory activities; antioxidant activities (metal chelating, ferric reducing, total antioxidant capacity), total phenol and flavonoid contents of the most active extracts were investigated. Moreover, some phenolic acids and flavonoids which were previously determined in *Inula* taxa were investigated for antidiabetic activity.

2. Materials and methods

2.1. Plant materials

Plants were collected in their flowering stages from different cities of Turkey. *Inula helenium* (L.) ssp. *turcoracemosa* Grierson and *I. montbretiana* DC. were collected near Ankara, *I. peacockiana* (Aitch. & Hemsl.) Krovin from Van, *I. thapsoides* (Bieb. ex Willd.) Sprengel ssp. *thapsoides* from Erzurum and *I. viscosa* (L.) Aiton from Isparta. Voucher specimens have been deposited in the Herbarium of Ankara University Faculty of Pharmacy under the herbarium codes of AEF 25193, AEF 25191, AEF 25124, AEF 25123 and AEF 26700, respectively.

2.2. Preparation of extracts and standards

Dried and milled flowers, leaves and roots of samples were extracted with water, methanol and ethyl acetate (5% *w*/*v*) by magnetic stirrer for 1 h (50 °C, 250 rpm). Extracts were then filtered from filter paper. Methanol (MeOH) and ethyl acetate (EtOAc) extracts were condensed by a rotary evaporator (Buchi-R200) and the aqueous extracts were freeze-dried. Yields of the extracts were calculated and given in Table 1. Phenolic compounds were purchased from Sigma (Germany): chlorogenic acid (C3878), caffeic acid (C0625), quercetin (Q0125), luteolin (L9283), rutin (R5143), and hispidulin (SML0582). Helenin was supplied from Roth (Roth 7677).

2.3. Assay for α-amylase inhibitory activity

The α-amylase inhibitory activity of the selected *Inula* species was determined by the method of Ali et al. (2006). Porcine pancreatic α-amylase type VI (EC 3.2.1.1, Sigma) was dissolved in distilled water. As substrate solution, potato starch (0.5%, *w*/*v*) in phosphate buffer (pH 6.9) was used. Experiments were carried out with three replicates.

Plant extract and pure compounds dissolved in DMSO and distilled water were mixed in a tube. The reaction was initiated by the addition of the enzyme solution. Then the tubes were incubated at 37 °C for 3 min. After the addition of substrate, the tubes were incubated at 37 °C for 5 min. Then, DNS (3,5-dinitrosalicylic acid) colour reagent solution was added to the mixture and put into a 85 °C heater. After 15 min, distilled water was added to the tubes and tubes were cooled. Absorbances of the mixtures were read at 540 nm. Acarbose was used as the positive control. The absorbance (*A*) due to maltose generated was calculated according to following formula: $A_{\text{Control or Sample}} = A_{\text{Test}} - A_{\text{Blank}}$.

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 % = [(Maltose $_{\text{Control}}$ − Maltose $_{\text{Sample}}$) / Maltose $_{\text{Control}}$)] × 100.

2.4. Assay for α-glucosidase inhibitory activity

α-Glucosidase type IV enzyme (Sigma Co., St. Louis, USA) was dissolved in phosphate buffer (0.5 M, pH 6.5). The enzyme solution, extracts and pure compounds were preincubated in a 96-well microtiter plate for 15 min at 37 °C. After that, the substrate solution [*p*-nitrophenyl-α-D-glucopyranoside (NPG), Sigma] 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 (Lam et al., 2008). Acarbose (Bayer Group, Turkey), a potent alpha-glucosidase inhibitor, was used as positive control. The inhibition percentage (%) was calculated by the equation: Inhibition % = $[(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$.

Some of the prepared EtOAc extracts could not be solved in the solvent system of the experiment. Thus, the activity of these extracts could not be tested and these extracts were mentioned as NT (not tested) in the concerning tables (Tables 2–4).

2.5. Metal chelating activity

The chelating activity of *Inula* extracts on Fe^{+2} was determined by the method of Dinis et al. (1994). Extracts were incubated with $FeCl_2$ (2 mM). The reaction was initiated by the addition of 0.2 mL of ferrozine (5 mM) and the total volume was adjusted to 4 mL with ethanol. After 10 min, the absorbance was measured at 562 nm. EDTA was used as a reference compound. The control contained $FeCl_2$ and ferrozine. The percentage of inhibition of the ferrozine-Fe^{+2} complex formation was calculated using this formula: Metal chelating activity (%) = $[(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$. Analyses were carried out in triplicate and the results were averaged.

2.6. Ferric-reducing antioxidant power

Different logarithmic concentrations of the extracts (3, 1, and 0.57 mg/mL) and ascorbic acid as reference were mixed with phosphate buffer (0.2 mol/L, pH 6.6) and $K_3Fe(CN)_6$. Tubes were incubated at 50 °C for 20 min, then trichloroacetic acid was added and the mixture was vortexed. Following centrifugation, the supernatant was mixed with same amount of distilled water and $FeCl_3$ and the absorbance at 700 nm was measured (Oyaizu, 1986). Analyses were run in three replicates and the results were averaged.