SUPPLEMENTARY MATERIAL

UHPLC-MS/MS phenolic profiling and *in vitro* antioxidant activities of *Inula graveolens* (L.) Desf.

Muzaffer Silinsin[1], Ercan Bursal[2,3]

[1]*Department of Biology, Institute of Science, Mus Alparslan University, Mus, Turkey*

[2]*Department of Nursing, School of Health, Mus Alparslan University, Mus, Turkey*

[3]*Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX, USA*

∗Author for Correspondence:

Dr. Ercan BURSAL

Texas Tech University, Department of Chemistry and Biochemistry, Box 41061, Lubbock, TX 79409-1061 TX, USA, Tel: 1 (806) 702 1220

E-mails: ercanbursal@gmail.com ; ercan.bursal@ttu.edu

Abstract

Inula graveolens (L.) Desf. is an annual aromatic herb which has various uses on alternative medicine in many region of the world. In this study, antioxidant activities of ethanol and water extracts of the plant leaves were determined by *in vitro* DPPH method and phenolic composition of the plant sample was determined by LC-MS/MS analysis. The results showed that chlorogenic acid, quinic acid, hyperoside, protocatechuic acid, and quercetin were the major phenolic compounds among the twenty seven standard compounds. The significant antioxidant capacity of the plant might related with the high abundance of phenolic compounds.

Keywords: antioxidant; *Inula graveolens*; LC-MS/MS; phenolic compounds; chlorogenic acid

Experimental

Plant material

The plant samples were collected from the area of Bingol University Campus that has 1150-1200 m altitude on 10 September 2015 by Dr. Omer Kilic from Bingol University. Also, Dr. Kilic carefully identified *I. graveolens* samples based on "The Flora of Turkey and East Aegean Islands, Volume 5" (Davis 1978, 54-73). The voucher specimens were numbered (5335) and deposited at the herbariums of both Hacettepe University and Bingol University. The plant leaves were dried on air, and powdered for using on extraction.

Chemicals

The standard phenolic compounds of LC-MS/MS that listed on Table 1 were purchased from Sigma-Aldrich (Germany) and Fluka (Germany). Also, the solvents, reagents and other chemicals that used for antioxidant methods were bought from Sigma-Aldrich (Germany).

Preparation of extracts

For LC MS/MS analysis, dried powder of the plant leaves (100 g) were three times extracted with 300 mL of methanol for 24 h at room temperature. The methanol solvent was removed with rotary evaporator under vacuum at room temperature (yield: 15.6 %). The dried samples were diluted to 1000 mg/L and filtrated with 0.2 µm microfiber.

For antioxidant studies, both ethanol and water extracts were prepared to investigate the function of solvents. The powder of air dried plant material was added to each distilled water

and etanol (1/10 : w/v), separately. The mixtures were stirred by magnetic stirrer during one day at room temperature and filtrated with filter paper. The water extract was lyophilised in a lyophiliser (Labconco, Freezone 1L) at 5 mm Hg at -50 °C to remove water solvent. The ethanol extract was evaporated with a rotary evaporator (Heidolph 94200, Bioblock Scientific) to remove ethanol solvent. Both lyophilized and evaporated samples were stored at -30 °C until used.

LC-MS/MS analysis

Analysis by LC−MS/MS was performed on a Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an ESI source. The LC instrument was equipped with binary pumps (LC-30AD), degasser (DGU-20A3R), column oven (CTO-10ASvp) and auto sampler (SIL-30AC). A reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 µm) C18 analytical column was used for chromatographic separation of organic compounds. A sample volume of 4 µL was injected during analysis. The flow rate was set at 0.5 mL/min, and solvent A was HPLC water containing 5 mM ammonium formate with 0.1% formic acid and solvent B was methanol containing 5 mM ammonium formate with 0.1% formic acid. The following flow gradient was used: 0% solvent B for 40 min, 20% solvent B for 90 min, 24% solvent B for 90 min, 24% solvent B for 40 min, 29% solvent B for 40 min. Data dependent acquisition experiment of investigated compounds was programmed to conduct three scan events. The first scan event was for quantitative purposes, the second and the third scan events were for confirmation. Electrospray ionization (ESI) conditions were set at 300 °C, 3 L/min nebulizing gas flow and 15 L/min drying gas flow. The column temperature was set at 40 °C (Ertas et al. 2015).

Antioxidant activity

The antioxidant activities of water and ethanol extracts of plant material evaluated by DPPH free radical scavenging method. First of all, different concentrations (10-30 µg/mL) of extracts and standard were prepared. Then, daily prepared DPPH radical solution (0.1 mM) was added to the each sample. The mixtures were left in the dark at room temperature for 30 min. The absorbance measurements of the mixtures were obtained at 517 nm by using a spectrophotometer (Shimadzu, UV-1800, Japan). The amount of DPPH free radicals were calculated in the presence of the extracts or standards at different concentrations (Turan et al. 2016).

FIGURE CAPTIONS

Figure S1. UHPLC-ESI-MS/MS chromatograms of standard phenolic compounds **(A)** and *I. graveolens* **(B)**

Figure S2. Antioxidant activity of *I. graveolens* extracts and standard antioxidants by DPPH method

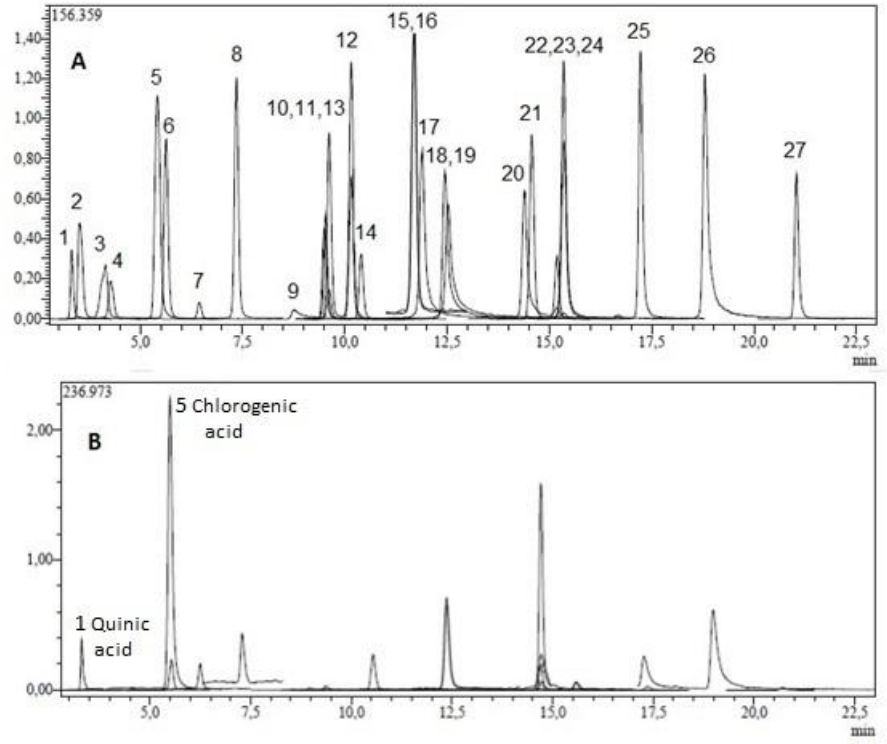

Figure S1

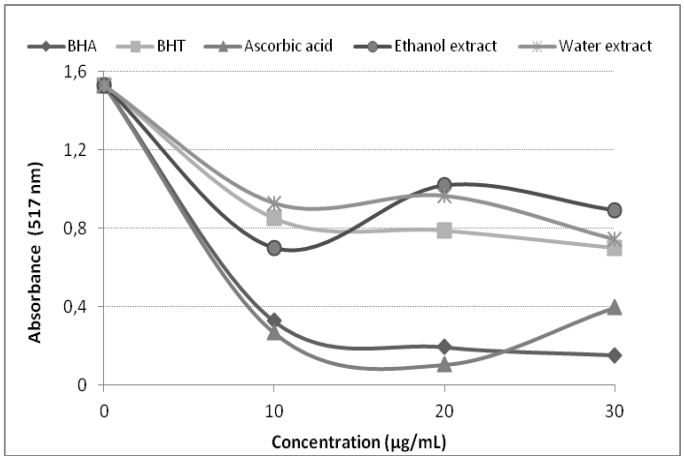

Figure S2