Supplementary File

Chemical composition and antioxidant activity of the essential oil and various extracts of *Inula graveolens* (L.) Desf.

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Extract preparation

The collected plant was washed with distilled water, dried in shade and powdered by a domestic blender. The powder of the plant (20 g) was extracted successively with 500 mL of different solvents in increasing order of polarity from chloroform, acetone, acetonitrile, ethanol and methanol by using Soxhlet extractor (Gherart, Germany) for 48 h at 40 ^{o}C. The extracts were then concentrated in vacuum at 40 ^{o}C using a rotary evaporator and kept it in dark at +4 ^{o}C.

Isolation of the essential oil

Aerial parts of the collected plant material was dried in the shade and ground in a grinder. The ground aerial parts were then submitted to water distillation for 3 h using a Clevenger apparatus to produce the essential oil in a yield of 1.2 % (v/w), based on the dry weight of the sample from I. graveolens. The oil was stored in the dark at +4 ^{o}C until used (Mahboubi, 2011).

GC-MS analysis conditions

GC–MS analyses were performed by using an Agilent-5973 Network System. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was HP-5 capillary column (30 m x 0.32 mm i.d., film thickness 0.25 μm). The carrier gas used was helium, at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 ^{o}C. One microliter essential oil in hexane (HPLC grade) was injected and analyzed with the column held initially at 60 ^{o}C for 2 min and then increased to 260 ^{o}C with a rate of 5 ^{o}C/min heating ramp and subsequently kept at 260 ^{o}C for 13 min. The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator. The identification of individual compounds was based on comparison Wiley 7 NIST and published data (Tepe et al., 2011).

Total antioxidant activity by β-Carotene–linoleic acid method

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Öztürk et al., 2007). A stock solution of β-carotene–linoleic acid mixture was prepared as follow: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade). 25 µL linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added with vigorous shaking; 2.5 mL of this reaction mixture was dispersed to test tubes and 0.5 mL of the extracts (2 mg/mL) in water were added and the emulsion system was incubated at 50 °C for to 2 h. The same procedure was repeated with the positive control butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and for blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of the absorbance was continued until color of β-carotene disappeared. The bleaching rate (R) of β-carotene was calculated according to the following formula :

$$R = \ln(a/b)/t \quad (1)$$

Where, ln=natural log, a=absorbance at time 0, b=absorbance at time t (120 min) (Dapkevicius et al., 1998). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the following formula :

$$AA = [(R_{control} - R_{Sample}) / R_{control}] \times 100 \quad (2)$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 2 mg/mL and blank consisting of only 0.5 mL water.

Scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured from the bleaching of purple colored methanol solution of

DPPH. The effect of water extracts on DPPH radical was estimated according to (Cheung et al., 2003). 1 mL of various concentrations (0.2-1.0 mg/mL) of the extracts in water was added to a 1 mL of DPPH radical solution in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed standing for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). Inhibition of free radical DPPH in percent (I %) was calculated in following way:

$$I\% = 100 \times (A_{Control} - A_{Sample}) / A_{Control} \quad (3)$$

Where, $A_{Control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{Sample} is the absorbance of the test compound. BHT and BHA were used as a control.

Reducing power

The reducing power was determined according to the method of (Cheung et al., 2003) with slight modification[9]. Each extract (0.2-1.0 mg/mL) in water (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized distilled water and 0.5 mL of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. BHT and BHA were used as a control.

Chelating effects on ferrous ions

The chelating effect was determined according to the method of (Oyaizu, 1986). Briefly, 1 mL (2 mg/mL) of the extracts in water was added to 1 mL of water and a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of

the solution was measured spectrophotometrically at 562 nm. The inhibition percentage of ferrozine-Fe2+ complex formation was calculated by using the formula given below:

$$\textit{Metal chelating effect (\%)} = [(A_{Control}-A_{Sample})/A_{Control}] \times 100 \quad (4)$$

Where $A_{Control}$ is the absorbance of control (The control contains FeCl2 and ferrozine, complex formation molecules) and A_{Sample} is the absorbance of the test compound. EDTA was used as a control.

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