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Antioxidant Activity of Some Furanocoumarins Isolated from *Heracleum persicum*

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Abstract

Antioxidants are important substances that possess the ability to protect the body from damage caused by free radical–induced oxidative stress. A variety of free-radical scavenging antioxidants exist within the body, many of which are derived from dietry sources such as fruits, vegetables, and teas. This paper describes a search for bioactive components in fruits of *Heracleum persicum* Desf. ex Fisher, which led to isolation of four furanocoumarins that exhibit antioxidant activity. The structure of the constituents were identified by ultraviolet, infrared, nuclear magnetic resonance, and mass spectrometry analyses in comparison with literature data. The isolated constituents were tested by linoleic acid peroxidation for their antioxidant activities and were found to be moderately active. Antioxidant activity of crude ethyl acetate extract was stronger than single isolated constituents.

Keywords: Antioxidant, free radical, furanocoumarins, *Heracleum persicum*, linoleic acid.

Introduction

It is widely accepted that some pathological events such as heart disease, chronic renal failure, diabetes mellitus, cancer, immune dysfunction, and aging are closely related to the peroxidation reactions in living organisms (Maxwell, 1995; Halliwell, 2000; Noguchi & Niki, 2000; Young & Woodside, 2001). Antioxidants are supposed to protect cell membranes against free-radical oxidative damages (Maxwell, 1995; Halliwell, 2000). There is growing interest to replace synthetic antioxidants by natural ones mostly found in plants (Velioglu et al., 1998). There is also a worldwide trend toward the use of natural additives in foods and cosmetics. For this reason, an extensive search for different types of antioxidants in various types of plants has been undertaken (Larson, 1988; Gazzani et al., 1998; Velioglu et al., 1998).

Heracleum persicum Desf. ex Fisher (Umbelliferae) (locally known as "Golpar") is an annual plant native to Iran with a wide distribution, especially in northern mountainous regions with an altitude ranging from 1500–2500 m (Parsa, 1948). Its fruit has traditionally been used as a spice, food additive, supplement, and for pickling. It is also recommended as a carminative (Amin, 1991).

A literature survey showed a few phytochemical and biological investigations including isolation of furanocoumarins from roots, leaves, and fruit of *Heracleum persicum* (Merijanian et al., 1980; Aynehchi et al., 1987). The current study was performed to isolate and identify the furanocoumarins of this plant and evaluate the antioxidant activity of these constituents by linoleic acid peroxidation.

Materials and Methods

Plant material

The fresh fruit of *Heracleum persicum* Desf. ex Fischer (Umbelliferae) was collected from Kandowan, a mountainous region (Alborz) of Mazandaran province (Iran) in June 2002 and was authenticated by the Herbal Museum, Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences (voucher no. 6541). The fruit was air-dried at room temperature and kept in an air-tight light-protected container.

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Chemicals

Linoleic acid and 1,3-diethy-2-thiobarbituric acid (DETBA) were obtained from Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Milwaukee, WI, USA), respectively. α-Tocopherol, sodium dodecyl sulfate (SDS), and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were from Merck and were of analytical grade.

Extraction procedure

The air-dried fruits were ground into fine powders and 1200 g was soaked in 2 L of hexane at room temperature overnight. The solvent was filtered, and the residue was macerated 4 more days with the same solvent. The solvent was filtered and evaporated to dryness under reduced pressure at 40 °C to give a hexane extract (100 g). Further extraction processes were carried out with chloroform, ethyl acetate, methanol, and water in order of increasing polarity to give 80, 25, 40 and 110 g of soluble fractions, respectively. The antioxidant activity of these fractions was tested against linoleic acid peroxidation. The ethyl acetate extract showed the highest activity. Therefore, the ethyl acetate extract was chromatographed on silica gel 60 PF254 (chloroform-ethyl acetate, 98.5/1.5), and four fractions were obtained. Crude fractions were further chromatographed on silica gel and crystallized to obtain purified compounds.

Identification of the isolated compounds was carried out by comparison of their spectroscopic data (m.p., MS, UV, IR, ^{1}H NMR, and ^{13}C NMR) with those reported in the literature. (Copies of original spectra are available from the author for correspondence.)

Instruments

Ultraviolet spectra were obtained on a Shimadzu (Kyoto, Japan) UV-160 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ using Varian 400 Unity *plus* spectrometer. Chemical shifts were in parts per million (ppm) from tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded with a Finigan TSQ-MAT 70 mass spectrometer at 70 eV. Fourier-transform infrared (FTIR) spectra were obtained with a Nicolet FTIR 550 spectrometer. Melting points were measured with a Reichert-Jung apparatus (Wein, Austria).

Measurement of antioxidant potential

The antioxidant activity of isolated compounds against peroxidation of linoleic acid was determined based on a method reported by Furuta et al. (1997). For a typical assay, 20 µl of each sample (three dilutions of each isolated compound) was mixed with 20 µl of 2 mg/ml linoleic acid in ethanol and incubated at 80 °C for 60 min. Incubated samples were cooled in an ice bath followed by addition of 200 µl of 20 mM BHT, 200 µl of 8% SDS, and 400 µl of distilled water. After mixing, a solution of 3.2 ml of 1.25 mM DETBA in sodium phosphate buffer (0.125 M, pH = 3.0) warmed to 50 °C was added, mixed, heated at 95 °C for 15 min, and cooled in an ice bath. Then, 4 ml of ethyl acetate was added to each tube, vortexed to extract the pink adduct from the aqueous phase, and centrifuged at 700 *g* for 10 min (F_1). A control, containing linoleic acid and other additives without antioxidants, representing 100% lipid peroxidation was also prepared (F_2). The fluorescence intensities of ethyl acetate layer (F_1) and control solution (F_2) were measured at an excitation wavelength of 515 nm and an emission wavelength of 555 nm in a spectrofluorimeter (Model RF-5000, Shimadzu) against their own blanks (F_3 and F_4, respectively) prepared as described above without linoleic acid. The antioxidant activity was calculated as the percent of peroxidation inhibition using the following equation (Okada & Okada, 1998):

$$\% \text{ of peroxidation inhibition} = [1-(F_1-F_3)/(F_2-F_4)] \times 100$$

All extracts and reference substance were assayed in triplicate, and the results were averaged. Percent inhibition versus log concentration was plotted, and the required concentration of sample for 50% inhibition was determined and expressed as the IC_{50} value.

Results and Discussion

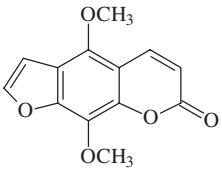
Structural determination of compounds

Preliminary studies showed that the antioxidant activity of *Heracleum persicum* is higher in the ethyl acetate extract. To prepare the active fractions, a solvent extraction method was carried out as described. Fractionation of the ethyl acetate extract of *Heracleum persicum* fruit led to isolation of four furanocoumarins. The structure of these furanocoumarins was determined by melting point (m.p.) and mass spectrometry (MS), ultraviolet (UV), infrared (IR), and nuclear magnetic resonance (NMR) spectral examinations and by comparison of the results with literature data (Elgamal et al., 1979; Harkar et al., 1984; Razdan et al., 1987; Reed & Moore, 1988; Kondo et al., 1990). Four furanocoumarins, pimpinellin (**1**), isopimpinellin (**2**), bergapten (**3**), and bakuchicin (**4**) (Fig. 1), were identified with the following characteristics:

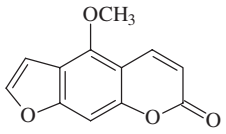
Compound (**1**): Isopimpinellin, golden yellow needles (ethyl acetate), m.p. 139–141 °C, R_f 0.45 (toluene-ether, 1 : 1 saturated with 10% acetic acid), *m*/*z* 246, ^{1}H NMR and ^{13}C NMR were in good agreement with published data (Elgamal et al., 1979; Razdan et al., 1987).

Compound (**2**): Pimpinellin, colorless needles (ethyl acetate), m.p. 115–117 °C, R_f 0.51 (toluene-ether, 1 : 1 saturated with 10% acetic acid), *m*/*z* 246, ^{1}H NMR was in good agreement with published data (Reed & Moore, 1988). ^{13}C NMR is shown in Fig. 2.

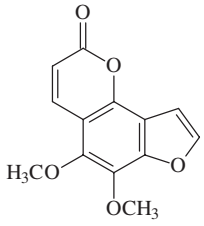
Compound (**3**): Bergapten, colorless needles (acetone), m.p. 176–178 °C, R_f 0.53 (toluene-ether, 1 : 1 saturated with 10%



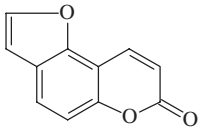
(**1**): Isopimpinellin



(**3**): Bergapten

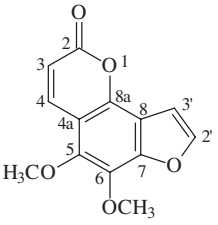


(**2**): Pimpinellin



(**4**): Bakuchicin

Figure 1. Chemical structures of isolated furanocoumarins from *Heracleum persicum.*



C-2	160.74	C-7	149.09
C-3	113.72	C-8	135.11
C-4	139.84	C-8a	143.21
C-4a	109.40	C-2'	145.34
C-5	144.47	C-3'	104.29
C-6	114.09	OCH_3 (C-5/6)	61.15/62.29

Figure 2. ^{13}C NMR spectral data of pimpinellin. (Chemical shifts in ppm relative to TMS. Solvent $CDCl_3$.)

acetic acid), *m*/*z* 216, ^1H NMR and ^{13}C NMR were in good agreement with published data (Elgamal et al., 1979; Harkar et al., 1984; Razdan et al., 1987).

Compound (**4**): Bakuchicin, colorless needles (acetone), m.p. 138–140 °C, R_f 0.64 (toluene-ether, 1 : 1 saturated with 10% acetic acid), *m*/*z* 186, ^1H NMR and ^{13}C NMR were in good agreement with published data (Kondo et al., 1990).

According to our literature survey, pimpinellin, isopimpinellin, and bergapten were previously reported from different parts of *Heracleum persicum* (Merijanian et al., 1980; Aynehchi et al., 1987), but this may be the first report on the presence of bakuchicin (**4**) in this species.

Antioxidant activity of isolated compounds

Unsaturated fatty acids in membrane lipids, especially linoleic acid, are most susceptible to oxidative reactions.

Table 1. Antioxidant activity of furanocoumarins.

Compounds	IC_{50} (μg)
Isopimpinellin	11.83 ± 0.04
Pimpinellin	17.16 ± 0.32
Bergapten	38.39 ± 2.44
Bakuchicin	44.03 ± 6.15

Inhibition of linoleic acid peroxidation has widely been used as a valuable procedure. In this study, the ability of isolated compounds to inhibit peroxidation of linoleic acid was determined by a procedure based on a sensitive method reported by Furuta et al. (1997). The effect of isolated furanocoumarins on peroxidation of linoleic acid was determined at different concentrations with three repetitions. Their 50% inhibitory concentrations are shown in Table 1. As displayed in this table, all four furanocoumarins presented a moderate antioxidant activity that was lower than that of α-tocopherol (IC_{50} = 0.60 μg). Among these compounds, isopimpinellin revealed the most potent activity with an IC_{50} value of 11.83 μg. IC_{50} values of the other constituents were 17.16 μg (pimpinellin), 38.39 μg (bergapten), and 44.03 μg (bekuchicin). Identification of these furanocoumarins was previously achieved (Elgamal et al., 1979; Merijanian et al., 1980; Harkar et al., 1984; Aynehchi et al., 1987; Razdan et al., 1987; Reed & Moore, 1988; Kondo et al., 1990), but no study on biological activity of these compounds has so far been reported.

The IC_{50} value of the crude ethyl acetate extract was about 0.1 μg, which was lower than all individual furanocoumarins. Therefore, it seems that the antioxidant activity of total extract of the fruit might present a better effect than single, isolated constituents. It may be presumed that popular use of the dried fruit powder as a spice among the Iranian population could have a protective effect against free-radical damage.

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