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Article

Chemical compositions and antioxidant activity of *Heracleum persicum* essential oil

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In this study essential oil of the aerial parts of *Heracleum persicum* a spice widely used in Iran was isolated by conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) techniques. The extraction yield was determined and the chemical compositions of essential oils were identified by the application of gas chromatography/mass spectrometry (GC/MS). The antioxidant activity was determined by two different methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and oven test methods. Although the main compounds of essential oils by the both extraction methods were similar, the essential oil extracted by HD with lower extraction efficiency showed more diverse compounds. The evaluation of antioxidant activity of the essential oil measured by delay in sunflower oil oxidation indicated that the antioxidant activity was dependent on the concentration which increased when higher concentrations of the essential oils were applied. The results of DPPH radical assay also indicated that the percentage of inhibition increased with increasing of essential oil concentration and IC_{50} value for essential oil extracted by MAHD method was obtained 1.25 mg/mL. Therefore the *Heracleum persicum* essential oil might be recommended for use as a flavoring agent and a source of natural antioxidants in functional foods, formulation of the supplements and in medicinal due to numerous pharmacological activities.

Keywords: *Heracleum persicum*/essential oil/chemical composition. *Heracleum persicum*/functional properties. *Heracleum persicum*/antioxidant activity.

INTRODUCTION

Heracleum persicum is a flowering plant and native to Iran that commonly known as "Golpar". It belongs to Apiaceae family in the order of Apiales that contains about 300 genera and more than 3000 species. *Heracleum* species probably originates from the Middle East, somewhere south of Caucasian, but has spread as an ornamental plant up to Northern Europe. *Heracleum* genus has 10 species in Iran (Asgarpanah *et al*., 2012).

The ripe fruits, leaves and young stems of *Heracleum persicum* are used as antiseptic, carminative, digestive and analgesia as well as flavoring in the Iranian folk medicine (Torbati *et al*., 2014). Also it has recently been shown to have antioxidant, anticonvulsant, anti-inflammatory and immunemodulatory activities (Asgarpanah *et al*., 2012).

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Among different methods for extraction of essential oils from spices, hydrodistillation is the common and most frequently used method (Morsy, 2015). However, in order to reduce the extraction time, the operation costs, possibly improve the yield and the quality of the essential oils, new approaches such as microwave-assisted extraction, supercritical fluid extraction and ultrasound-assisted extraction have also been in consideration (Memarzadeh, Ghasemi Pirbalouti, Adibnejad, 2015).

In this regards some advances in using microwave have resulted in a number of techniques such as microwave steam diffusion (Farhat *et al*., 2011), microwave hydrodiffusion and gravity (Vian *et al*., 2008), microwave dry distillation (Tigrine Korjani, Meklati, Chemat, 2006) and solvent free microwave extraction (Lucchesi *et al*., 2007). Some recently studies (Golmakani, Rezaei, 2008; Perino Issartier *et al*., 2013; Stashenko, Jaramillo, Martinez, 2004; Tigrine Kordjani, Meklati, Chemat, 2006) have successfully utilized a microwave oven for the extraction of active components from medicinal plants.

Therefore the aim of this research was the comparison of the chemical compositions and antioxidant activity of *Heracleum persicum* essential oil extracted by conventional hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) techniques.

MATERIALS AND METHODS

Material

Dried aerial parts of *Heracleum persicum* a spice widely used in Iran were supplied from Tehran market. The identity of the *Heracleum* was certificated by top experts from Herbarium of the Islamic Azad University, Science and Research Branch. Aerial parts of *Heracleum persicum* were ground in a mill (Triplex, France), passed through a sieve of 0.5 mm mesh and the powder obtained was stored in plastic bags, in darkness at 4 °C. Refined sunflower seed oil without added synthetic antioxidant was purchased from Behshahr Oil Company of Tehran. All the chemicals used were of analytical grade, purchased from Merck Chemical Company of Germany.

Isolation of essential oil

Hydrodistillation

Powder of aerial parts of *Heracleum persicum* were hydrodistillated for 3 h using a Clevenger-type apparatus according to the standard procedure. The essential oil volume was measured directly in the extraction burette. The obtained essential oils were dried with anhydrous sodium sulphate and then stored in a sealed dark vials at 4°C until further analysis. Yield percentage was calculated as volume (mL) of essential oil per 100 g of plant dry matter (Jordán *et al*., 2009).

Microwave-assisted hydrodistillation (MAHD)

A domestic microwave oven (micro SYNTH, 2450 MHz) was modified for MAHD operation. The interior cavity of the microwave oven was 29 × 37 × 40 cm. Flat bottom flask having a capacity of 500 mL was placed inside the cavity for the MAHD experiments. 20gr of powdered samples soaked in 100 mL of distilled water for 30 minutes were placed in a 500 mL flask. The flask was setup within the microwave oven cavity and a condenser was used on the top, outside the oven. The microwave oven was operated at 600 power level for a period of 30 min which no more essential oil was obtained. The extracted essential oils were collected, dehydrated with anhydrous sodium sulfate and kept in a sealed dark vials at 4 °C (Karakaya *et al*., 2014).

Chemical composition of essential oils

The chemical compositions of essential oils obtained from aerial parts of *Heracleum persicum* by using hydrodistillation and microwave-assisted hydrodistillation methods were identified by the application of gas chromatography/mass spectrometry (GC/MS). Samples were analyzed by using an Agilent Hp-6890 gas chromatograph (Agilent Technologies, USA) with a Hp-1 Methyl silicone capillary column (60 m×0.32 mm×0.25 µm film thickness) linked to Hp-5973 mass spectrometry detector. Oven temperature was set as 60 °C for 3 min initially and increased to 230 °C, at a rate of 7 °C/min and subsequently held isothermal for 3min. Injector temperature was set as 250 °C. Helium was used as carrier gas at a flow rate of 1 mL/min and 1 µL sample was injected manually in the split mode. Ionization of sample compounds was performed in the ET mode (70 ev). The identification of compounds was performed by comparison of their mass spectral pattern and their linear retention indices based on a homologous series of normal alkanes (C8–C20) with those of authentic references and the Wiley 257 mass spectra database.

Antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging activities of essential oils were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by El Ouariachi *et al*. (2014). Antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. Various concentrations of essential oils were added to DPPH radical solution in ethanol. The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm by using a spectrophotometer (Cary 50 Scan UV-Visible Spectrophotometer). The radical scavenging activity was expressed as percentage of inhibition (I%) according to the following equation:

$$I(\%)=[1-(A_{sample}/A_{control})]\times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound.

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

Oven test method

The essential oil extracted by MAHD method was added to 100 g sunflower seed oil at the concentrations of 0.02, 0.04, 0.06, 0.08 and 0.1% (w/w) to examine their antioxidant activity. The antioxidant activity of extracts was compared with tertiary butylhydroquinone (TBHQ) at the concentration of 0.01% (w/w). All the samples were placed in the oven at 90 ºC and the peroxide values were determined every 24, 48, 72 and 96 h according to AOCS method, Cd 8–53 by dissolving oil in 3:2 acetic acid-chloroform solution and titration with 0.01 N sodium thiosulfate solution in the presence of potassium iodide and starch indicator (Firestone, 1994).

Statistical Analysis

All the experiments and measurements were carried out in triplicate order. The data were statistically analyzed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Analyses of variance were performed by application of the ANOVA procedure. Significant differences between the means were determined using Duncan's multiple range test.

RESULTS AND DISCUSSION

The essential oil extraction by MAHD method started at much earlier time than HD method (5min and 35 min respectively). The process continued until no more essential oil was obtained. Full extraction of essential oils was achieved within the first 30 min of operation with MAHD method. In the case of HD method, a time period of at least 3 h was necessary for such purpose.

It seems that it is due to the more efficient heat flow involved with microwaves. Unlike the classical conductive heating method, microwave can heat the entire sample almost simultaneously and at a higher rate (Golmakani, Rezaei, 2008).

The results indicated that the yield of essential oil extracted by MAHD (2.0%, v/w) in shorter time of operation was greater than that obtained by HD (1.7%, v/w) that means a substantial saving in time and operating cost.

Chemical compositions of the essential oils extracted by both MAHD and HD methods are presented in Table I.

Twenty constituents were identified by GC/MS of essential oil isolated by HD method that the predominant compounds were hexyl butanoate (28.32%), heptyl isobutanoate (24.05%), hexyl 2-methylbutanoate (8.24%), phenyl ethyl 3-methyl butanoate (5.41%), hexyl acetate (4.47%), isopropyl-3-methyl butyrate (2.91%), γ-terpinene (2.90%), octenol butanoate (2.87%), isopropyl-2-methyl butyrate (2.66%) and cymene (2.38%), accounted for 84.21% of the essential oil.

TABLE I - Chemical compositions of *Heracleum persicum* essential oils extracted by MAHD and HD methods

Composition	Method		
	HD		MAHD
	KI (Kovats Index)	Amount (%)	Amount (%)
Cyclohexene	831	0.28	8.99
Methyl pentanoate	877	0.43	-
Isopropyl-2-methyl butyrate	885	2.66	-
Isopropyl-3-methyl butyrate	901	2.91	-
Butyl butanoate	994	0.42	-
Octanal	998	0.41	3.79
Penthyl propanoate	1007	0.57	-
Isoamyl isobutyrate	1009	4.47	-
Cymene	1026	2.38	-
Limonene	1029	1.02	-
Terpinene(γ)	1059	2.90	-
Hexyl butanoate	1192	28.32	-
Caranone	1200	0.73	-
Hexyl 2- Methyl butanoate	1236	8.24	1.52
Heptyl isobutanoate	1250	24.05	78.50
Anethole	1284	0.41	-
Octenol butanoate	1388	2.87	1.36
Menthyl lactate	1469	0.50	1.98
Phenyl ethyl 3-methyl butanoate	1491	5.41	2.06
Cedrene-3-one	1645	0.24	-

The results indicated that the main constituents of the essential oil extracted by MAHD method were heptyl isobutanoate (78.50%), cyclohexene (8.99%), octanal (3.79%), phenyl ethyl 3-methyl butanoate (2.01%).

Although the main compounds of essential oils extracted by the both extraction methods were similar, essential oil extracted by HD with lower extraction efficiency showed more diverse compounds that might be due to the formation of new compounds by oxidation, glycoside hydrolysis, esterification and other processes in the longer time of extraction (Ghasemi Pirbalouti, Mahdad, Craker, 2013), also it means that the MAHD method is more selective than conventional hydrodistillation because does not involve in any deterioration of the extracted components and minimizes the risk of compound degradation due to the extraction rate (Memarzadeh, Ghasemi Pirbalouti, Adibnejad, 2015), therefore it might be introduced as a safe method for the extraction of essential oils.

Various techniques are available to evaluate the antioxidant activity of specific compounds or complex mixtures such as essential oils; however a single procedure cannot identify all possible mechanisms characterizing an antioxidant (Nikolic *et al*., 2013). Therefore, in the present study, two different methods were conducted in order to evaluate the antioxidant activities of *Heracleum persicum* essential oil; DPPH free radical scavenging assay and oil stability by the oven test method.

The results of DPPH radical-scavenging activity assay (Figure 1) indicated that the percentage of inhibition increased with increasing of essential oil concentration and IC_{50} values for essential oils extracted by HD and MAHD methods were obtained 1.43 mg/mL and 1.25 mg/mL respectively which is weaker than that reported by Coruh, Sagdicoglu and Ozgokce (2007).

Due to the extraction rate, efficiency, IC_{50} value and chemical composition of the essential oil obtained by

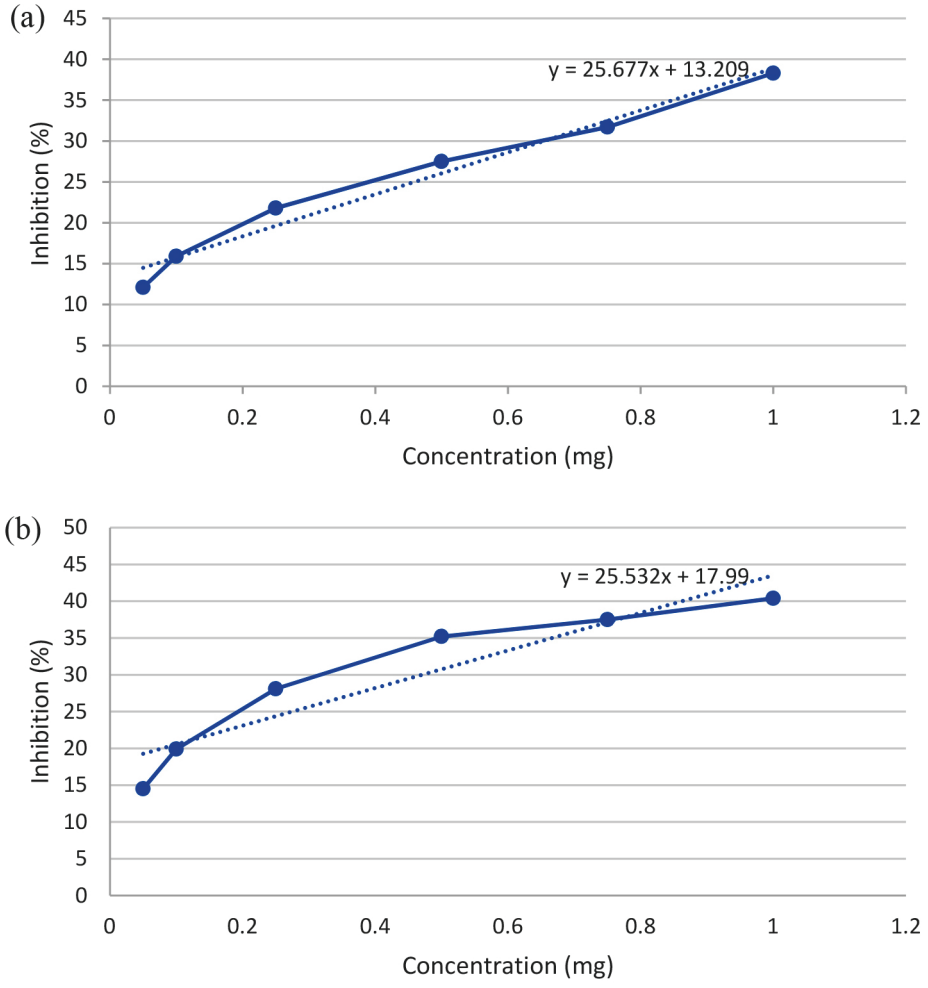

FIGURE 1 - DPPH radical scavenging activity; (a) essential oil extracted by HD method, (b) essential oil extracted by MAHD method. IC_{50} value for Ascorbic acid as control was obtained 0.06 mg/mL.

TABLE II - Peroxide values of sunflower seed oil with different concentrations of *Heracleum persicum* essential oil at 90ºC (meq/kg oil)*

Treatment	Time of heating				
	0 h	24 h	48 h	72 h	96 h
Control	0.05±0.00	17.91±0.02 [a]	39.12±0.05 [a]	61.82±0.10 [a]	79.84±0.03 [a]
TBHQ (0.01%)	0.05±0.00	3.00±0.05 [g]	7.11±0.08 [g]	16.56±0.05 [g]	29.00±0.05 [g]
MAHD (0.02%)	0.05±0.00	16.91±0.00 [b]	32.00±0.01 [b]	47.86±0.01 [b]	66.61±0.07 [b]
MAHD (0.04%)	0.05±0.00	13.86±0.07 [c]	29.97±0.00 [c]	44.50±0.03 [c]	57.21±0.03 [c]
MAHD (0.06%)	0.05±0.00	10.97±0.24 [f]	27.36±0.00 [d]	33.81±0.12 [d]	48.07±0.00 [d]
MAHD (0.08%)	0.05±0.00	11.40±0.16 [e]	25.90±0.05 [e]	28.54±0.05 [f]	46.53±0.14 [e]
MAHD (0.10%)	0.05±0.00	11.92±0.08 [d]	24.03±0.02 [f]	29.73±0.05 [e]	42.66±0.01 [f]

* The values are expressed as means ± standard deviation, n = 3. Different letters in each column indicate significant differences ($P< 0.05$).

MAHD method, oven test method was only carried out on this essential oil.

The effect of *Heracleum persicum* essential oil and TBHQ on sunflower seed oil oxidation are shown in Table II. Peroxide value of fresh sunflower oil was 0.05 meq/kg, which has increased as the result of oxidation. Sunflower oil with added TBHQ showed the lowest peroxide value after 96 h at 90 ºC as compared with other treatments, which might be due to the fact that TBHQ is a potent antioxidant particularly at accelerated oven test which is carried out at high temperature.

The results shown in Table II indicate that all used concentrations of essential oil decreased the rate of oxidation of sunflower oil and significant differences were found between all treatments ($p<0.05$). The evaluation of antioxidant activity of the essential oil measured by delay in sunflower oil oxidation also indicated that the antioxidant activity was dependent on the concentration which increased when higher concentrations of the essential oils were applied. Increasing the concentration of *Heracleum persicum* essential oil showed lower rate of oxidation and consequently lower peroxide values were obtained. The results indicated that the greatest increase in stability was obtained when sunflower oil treated with 0.1% (V/W) concentration of essential oil.

The limitations of this study include collecting plants due to its diversity, the possibility of contamination with pesticide and its toxicity.

The *Heracleum persicum* essential oil might be recommended for use as a flavoring agent and a source of natural antioxidants in functional foods, formulation of the supplements and in medicinal due to numerous pharmacological activities.

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