# Antioxidant Activity of Essential Oil from Black Zira (*Bunium persicum* Boiss.) Obtained by Microwave-assisted Hydrodistillation

S. Mazidi<sup>1</sup>, K. Rezaei<sup>2\*</sup>, M. T. Golmakani<sup>3</sup>, A. Sharifan<sup>1</sup>, and Sh. Rezazadeh<sup>4</sup>

## ABSTRACT

Microwave-assisted hydrodistillation (MAHD) at three levels of microwave power (180, 360, and 540 W) and the traditional hydrodistillation (HD) were applied to obtain essential oils from *Bunium persicum* Boiss. (Black Zira). MAHD at 540 W started much earlier than that of HD (4 min vs. 38 min, respectively). By the time the extraction of essential oils started with HD, almost 50% of the total essential oils (2.15%, w/w yield) had been extracted with MAHD at 540 W. Analysis of the essential oils using gas chromatography-mass spectrometry showed that  $\gamma$ -terpinene (28.16-31.13%, w/w), cuminaldehyde (24.85-29.20%),  $\rho$ -cymene (14.67-16.50%) and limonene (6.13-8.28%) were their main constituents, with a similar composition both after HD and MAHD extraction. The antioxidant activity (reported as IC<sub>50</sub>) of essential oil extracted by HD was 9.31 mg ml<sup>-1</sup> and those of MAHD at 180, 360, and 540 W were 8.62, 8.79, and 6.45 mg ml<sup>-1</sup>, respectively. Microwave irradiation did not cause any adverse effect on the antioxidant activities of the extracted essential oils, therefore, it can be used as a good alternative method to obtain essential oils from *B. persicum*.

Keywords: Bunium persicum, Black Zira, DPPH<sup>o</sup>, Essential oil, Microwave-assisted hydrodistillation.

# INTRODUCTION

*Bunium persicum* Boiss., commonly known as Black Zira, is a member of Apiaceae family and is an important aromatic perennial plant that naturally grows in Iran (Azizi *et al.*, 2009). From the medicinal point of view, *B. persicum* is used as stimulants and carminatives, and it seems also useful in treating diarrhea and dyspepsia (Baser *et al.*, 1997). The seeds are consumed widely as a condiment and as a traditional flavoring agent in a number of ethnic cuisines and also in food industries. The seeds of *B. persicum* are very popular seasoning for meat-based dishes in Central Asia (Karim *et al.*, 1977; Foroumadi *et al.*, 2002). Moreover, there are several studies in the literature related to the antimicrobial and antioxidant properties of essential oil from this plant species (Oroojalian *et al.*, 2010; Shahsavari *et al.*, 2008).

Because of the growing interest of consumers in natural ingredients and the increasing concern about consumption of synthetic additives, using essential oils and their constituents as functional components

<sup>&</sup>lt;sup>1</sup> Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Islamic Republic of Iran.

<sup>&</sup>lt;sup>2</sup> Department of Food Science, Engineering and Technology, University of Tehran, P. O. Box: 31587-77871, Karaj, Islamic Republic of Iran.

<sup>\*</sup> Corresponding author; email: krezaee@ut.ac.ir

<sup>&</sup>lt;sup>3</sup> Department of Food Science and Technology, School of Agriculture, Shiraz University, Shiraz, Islamic Republic of Iran.

<sup>&</sup>lt;sup>4</sup> Institute of Medicinal Plants (ACECR), Tehran, Islamic Republic of Iran.

in foods, drinks, and cosmetics deserves particular attention (Sacchetti et al., 2005; Gholivand et al., 2010). Among several extraction methods for the separation of volatile compounds from plant raw materials, hydrodistillation (HD), steam maceration. destructive distillation. distillation, and expression are the ones commonly used. However, low extraction efficiencies and long extraction times are the major concerns in using the abovementioned methods (Wang and Weller, 2006). Consequently, more innovative and rapid techniques, such as supercritical fluid extraction (Pourmortazavi et al., 2005), ultrasound-assisted extraction (Wang and Weller, 2006), and microwave-assisted extraction (MAE) (Wang et al., 2006) have been investigated. The advantages of using microwave heating in comparison with conventional methods include a shorter extraction time, faster energy transfer, reduced thermal gradients within the matrix, and higher quality and quantity of the extract (Eskilsson and Bjorklund, 2000; Ondruschka and Asghari, 2006). Microwave-assisted hydrodistillation (MAHD) is a process that uses microwave energy and water to extract the target compounds from medicinal plants/herbs. Although there are many studies reporting the extraction of essential oil from B. persicum, none of them are based on the use of microwave energy. Therefore, the aim of this study was to use the MAHD technique for extraction of essential oils from dried B. persicum seeds and to compare their compositions and antioxidant activities with those of the conventional HD.

## MATERIALS AND METHODS

## Chemicals

The 2,2-diphenyl-1-picrylhydrazyl (DPPH°), butylated hydroxyl toluene (BHT), and analytical grade solvents were supplied by Sigma-Aldrich (St. Louis, MO, USA).

#### **Plant Materials**

The dried seeds of B. persicum were obtained in July 2009 from Birjand region, located in the Southern Khorasan Province (Eastern Iran). The identity of the genus Bunium was certified by senior experts from Pharmacy Department of the University of Tehran, Iran. The certified species was kept in a dark and cold room until used for the experiments, shortly after storage. The moisture contents of the seeds were measured in triplicate according to AACC (1983) method 44-19, using a laboratory oven at 105°C until constant weight was achieved. The measured moisture content was 6.5%, w/w. All values are reported on a moisture-free basis.

# Microwave-assisted Hydrodistillation

MAHD was performed using a modified microwave oven (MC175; AEG, Germany). Figure 1 shows the schematic representation of the MAHD apparatus used in this study. The oven was operated at 2.45 GHz with a maximum delivered power of 900W, variable in 90 W increments. In a typical MAHD procedure performed at atmospheric pressure, 25.0 g of dried B. persicum seeds were heated in 250 ml of water for 4 hours in the microwave at the selected power levels of 180, 360, and 540 W (preliminary experiments showed that applying higher microwave power level was not practical considering the scale of the work). During the first and second 30 minutes, the collected essential oil was decanted from the condensate in 10- and 15-minutes intervals, respectively. After 60 minutes of operation, decantation of the essential oil was performed every 30-minutes up to 4 hours. To remove water, the extracted essential oil was then dried over anhydrous sodium sulfate, weighed, and stored in ambered vials at 4°C until analysis.

## Hydrodistillation Using the Conventional Clevenger Apparatus

HD was carried out essentially as reported for MAHD. However, an electric mantle heater (TG 500, Electrothermal Engineering Ltd. Iran, 250W) was used instead of microwave oven. During the first 60 minutes, the collected essential oil was decanted from the condensate in 15-minutes intervals. After the 60 minutes, decantation of the essential oil was performed every 30minutes, up to 4 hours.

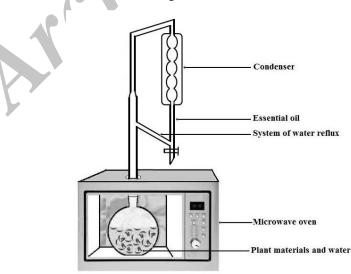
## **Physical Constants**

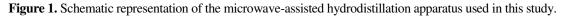
Specific gravity, refractive index, and color of the essential oils extracted from *B. persicum* (by both HD and MAHD) were measured according to a method suggested by Food Chemical Codex (FCC, 1996). Specific gravity was measured at 25 °C. Refractive index was measured at 20 °C.

# Gas Chromatography and Gas Chromatography-Mass Spectrometry conditions

To identify the components of the extracted essential oils, a gas

chromatography (GC) system (HP 6890N, Hewlett Packard, Palo Alto, CA, USA) coupled to a mass spectrometry (MS) detector (5973N; Agilent Technologies, Wilmington, DE) was used. An HP5MS column (30 m×0.25 mm and 0.25 µm film thickness) was used for the separation of the compounds. Temperature programming was as follows: a hold at 50°C for 5 minutes, a ramp of 3 °C min<sup>-1</sup> to 240°C, another ramp of 15 °C min<sup>-1</sup> to 300°C and a final hold at 300°C for 3 minutes. Helium was used as the carrier gas at a flow rate of 0.8 ml min<sup>-1</sup>. A splitted injection (at 1:10 ratio) was used to introduce the sample  $(1.0 \ \mu l)$ . Injection temperature was set at 290°C. Electron impact (at 70 eV) was used for the mass spectrometry ionization purposes. The compounds were identified by comparing their GC retention indices (I) determined with reference to a homologous series of C<sub>9</sub>- $C_{17}$  normal alkanes. Identifications of the compounds were confirmed by comparing their mass spectral fragmentation patterns with those stored in the MS database of US National Institute of Standards and Technology (NIST), Wiley libraries, and with literature data. A GC system (Younglin Acm 600, Seoul, South Korea) equipped with an HP5MS column (30 m×0.25 mm and 0.25 µm film thickness) and a flame ionization detector was used for the





quantitative determination of the identified compounds based on the relative area percents of the identified compounds.

## Antioxidant Activity: DPPH<sup>o</sup> Radical Scavenging Activity

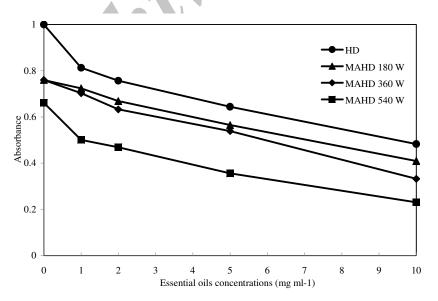
The free radical-scavenging activities of essential oil samples were measured using DPPH° as described by Brand-Williams et al. (1995). The effect of antioxidant on DPPH radical is a result of antioxidant hydrogen donating ability or radical-scavenging activity. Mixing the DPPH° solution with a substrate used for donating hydrogen atom leads to the reduced form (non radical DPPH) of this reagent with simultaneous change of the violet color to pale yellow (Ozkan et al., 2010; Ayoughi et al., 2011). DPPH° scavenging activity is shown by IC<sub>50</sub> value, defined as the concentration of the antioxidant required for the 50% loss of the DPPH° activity. Four ml of various concentrations of the samples (1, 2, 5)and 10 mg ml<sup>-1</sup>) in ethanol was added to 2 ml of 0.2 mM ethanol solution of DPPH°. The mixtures were shaken vigorously and left to stand at room temperature for 60 minutes in the dark. Then, the absorbance values were recorded at 517 nm against the blank. Inhibition percent of DPPH<sup>o</sup> (I%) was determined according to the following expression:

#### $I\% = ((A_c - A_s) / A_c) \times 100$

Where,  $A_c$  is the absorbance of the control reaction (containing all the reagents except for the test sample) and  $A_s$  is the absorbance of the sample after 60 minutes. The sample concentration providing 50% inhibitions (IC<sub>50</sub>) were determined from the equation of plotted inhibition curves. IC<sub>50</sub> values are shown in Table 2. Also, Figure 2 shows the DPPH scavenging activities of different concentrations of essential oils. All tests were carried out in duplicate and BHT was used as a positive control for antioxidant properties.

# **Statistical Analysis**

All extractions with HD and MAHD were performed in duplicate. A general linear model (GLM) procedure from SAS (Statistical Analysis Software, version 9.1; SAS Institute Inc. Cary, NC) was used for the comparison among the means.



**Figure 2.** Changes in the absorbance values of DPPH<sup>o</sup> solutions with different concentrations of essential oils (1, 2, 5, and 10 mg ml<sup>-1</sup>) from *B. persicum* Boiss. obtained by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) at 180, 360, and 540 W microwave power levels.

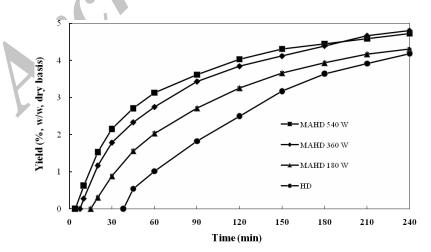
#### **RESULTS AND DISCUSSION**

## Comparison of the Effect of Extraction Methods on the Oil Yield

Figure 3 compares the extraction behaviors depending on the different conditions used. For both HD and MAHD, the extraction temperature was equal to the boiling point of water under the conditions of the study ( $\sim 100^{\circ}$ C). To reach such temperature level, where the actual distillation started, it was necessary to heat the samples for only 15.0, 7.5, and 4.0 minutes with MAHD at 180, 360, and 540W, respectively, while 38 minutes was necessary in the case of HD. This was due to the more efficient microwave heat flow. According to Figure 2, the extraction yield was generally improved by raising microwave power level from 180 to 540W. At higher levels of microwave power, oil-containing glands were possibly disrupted more rapidly resulting in a reduced process time (Iriti et al., 2006). This is in agreement with the findings of Rezvanpanah et al. (2008), who extracted essential oils from Satureja hortensis and Satureja montana using MAHD, where the extraction time was reduced significantly when the microwave power was changed from 220 to 660 W. The difference in the extraction yield at 180 and 540 W seemed to be more

pronounced during the first 2 hours of extraction. The required time to reach the boiling point of water at 540 W was nearly one-fourth of that at 180 W. After 20 minutes of operation, the extraction yield at 540 W (1.53%, w/w) was about five times more than that at 180 W (0.31%, w/w). In addition, the extraction yield for 540 W after 45 minutes of operation was the same as that obtained in 60 minutes at 360 W. Therefore, it can be concluded that extraction process at the highest power level studied here (540 W) was the best in view of saving time.

In the current study, by the time the extraction of essential oils started with HD at 30 minutes, almost 50% of the total essential oil (~2.15%, w/w) was extracted with MAHD at 540 W. Such level of extraction yield for MAHD in 30 minutes was even higher than that obtained after 90 minutes of operation by the traditional HD (1.82%, w/w). Again, a 60 minutes extraction with MAHD at 540 W provided a yield similar to that of HD after 150 minutes. These results are in good agreement with the findings of Stashenko et al. (2004) for Colombian Xylopia aromatica. They found that for the same extraction yield, the time required for MAHD was one-fourth of that for HD. Golmakani and Rezaei (2008a, b) also reported similar findings for the extraction yield of essential oils from Thymus vulgaris L. and Zataria multiflora Boiss. obtained by HD



**Figure 3.** Comparison of hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) at three different power levels (180, 360, and 540 W) in the extraction of essential oils from *Bunium persicum* Boiss.

and MAHD.

Final extraction yield of HD after 240 minutes (4.18%, w/w) was statistically similar to those obtained by MAHD at 180, 360, and 540 W after 210, 150, and 120 minutes, respectively. As final extraction yield results indicated, MAHD could decrease the time required for obtaining the same amount of essential oil by about 50% compared to HD, i.e. 120 instead of 240 minutes.

## **Evaluation of Physical Properties**

Physical properties (including specific gravities, refractive indices and appearances) of *B. persicum* essential oils extracted by MAHD at different power levels and those of essential oils extracted by HD are shown in Table 1. Results of this study indicated that the specific gravities and refractive indices of essential oils isolated by MAHD and HD were very similar. However, the colors of the essential oils extracted by MAHD at the three different power levels were somewhat lighter than that obtained by Golmakani and Rezaei (2008b) HD. reported similar results on the physical properties of essential oils from Zataria multiflora Boiss.

# **Compositions of Essential Oils**

The compositions of essential oils obtained by MAHD at the three levels of microwave power in the current study and HD are shown in Table 2. The main components of essential oils were  $\gamma$ -

terpinene and cuminaldehyde (compounds 9 and 13, respectively) followed by  $\rho$ -cymene and limonene (compounds 7 and 8, respectively). Similar results were reported by Foroumadi et al. (2002). Except for cuminaldehyde, whose content was significantly higher in the case of MAHD, the other main components of essential oils extracted by HD and those extracted by MAHD at 180, 360, and 540W were similar. In another study, Özek et al. (2005) extracted essential oils of three endemic Turkish Heracleum species by different techniques. Their results indicated some quantitative differences among some of the components. The extracted MAHDextracted oils showed slightly lower amounts of octyl acetate (59%) compared to those extracted by HD and other extraction techniques (93.7–94.9%). Instead, some other compounds were extracted at higher levels when using MAHD.

Compared to HD, no new compound was found in the essential oils extracted by MAHD in the current study. These results indicated that using microwave did not influence the quality of the extracted essential oil, but the extraction time was shorter. Similar findings were reported by Golmakani and Rezaei (2008a) for the compositions of essential oils extracted with HD and MAHD from Thymus vulgaris L. Our results are in agreement with those found by Wang et al. (2006) as well, who showed that the composition of Cuminum cyminum L. and Zanthoxylum bungeanum Maxim. essential oils extracted by HD and microwave-assisted extraction were similar.

**Table 1.** Physical properties of *B. persicum* essential oils extracted by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) at three levels of microwave power (180, 360, and 540 W).

	Extraction methods					
Physical properties	HD	MAHD 180 W	MAHD 360 W	MAHD 540 W		
Specific gravity (25°C)	$0.902^{a}$	0.907 <sup>a</sup>	0.918 <sup>a</sup>	0.929 <sup>a</sup>		
Refractive index (20°C)	1.4915 <sup>a</sup>	1.4970 <sup>a</sup>	1.4945 <sup>a</sup>	1.4955 <sup>a</sup>		
Appearance	Yellow	Pale yellow	Pale yellow	Pale yellow		

<sup>\*</sup> Letter "a" indicates that means in each row are not significantly different (P > 0.05).

					Relative pea	k area (%)*	
Peak No.	RT (Min)	Compound	RI	HD	MAHD (180 W)	MAHD (360 W)	MAHD (540 W)
1	10.54	α-Thujene	925	$0.32 \pm 0.07^{a^{**}}$	$0.18 \pm 0.01^{b}$	0.16±0.06 <sup>b</sup>	$0.11 \pm 0.02^{b}$
2	10.8	a –Pinene	930	1.59±0.24ª	$1.00 \pm 0.06^{b}$	0.87±0.04 °	0.63±0.06 <sup>c</sup>
3	11.4	Camphene	943	$0.07 \pm 0.01^{a}$	$0.05 \pm 0.00^{b}$	0.04±0.00°	$0.03 \pm 0.00^{\circ}$
4	12.94	$\beta$ -Pinene	975	3.26±0.43 <sup>a</sup>	2.73±0.20 <sup>b</sup>	2.51±0.01 <sup>b, c</sup>	2.14±0.27°
5	13.67	β-Myrcene	990	$0.74 \pm 0.08^{a}$	$0.64 \pm 0.05^{b}$	$0.62 \pm 0.03^{b}$	$0.59 \pm 0.04^{b}$
6	14.86	$\alpha$ –Terpinene	1014	$0.04 \pm 0.02^{b}$	0.13±0.06 <sup>a</sup>	$0.03 \pm 0.01^{b}$	$0.04 \pm 0.00^{b}$
7	15.74	$\rho$ -Cymene	1031	16.25±1.58 <sup>a, b</sup>	14.67±0.94 <sup>b</sup>	16.50±0.52 <sup>a</sup>	15.03±0.32 <sup>a, b</sup>
8	16.16	Limonene	1040	$7.57 \pm 0.60^{a}$	8.28±0.33ª	6.13±1.00 <sup>a</sup>	$7.45 \pm 1.44^{a}$
9	17.53	γ-terpinene	1067	31.13±1.65 <sup>a</sup>	28.22±1.49 <sup>a, b</sup>	28.16±0.23 <sup>b</sup>	$28.41 \pm 1.42^{a, b}$
10	18.95	α-Terpinolene	1095	1.17±0.11 <sup>a</sup>	$0.88 \pm 0.02^{b}$	0.98±0.12 <sup>b</sup>	$0.91 \pm 0.00^{b}$
11	22.46	Borneol	1166	$0.59 \pm 0.01^{b}$	$0.65 \pm 0.00^{a}$	$0.60 \pm 0.03^{b}$	$0.62 \pm 0.02^{a,b}$
12	23.09	Terpinene-4-ol	1179	1.43±0.06 <sup>b</sup>	$1.80\pm0.06^{a}$	1.55±0.01 <sup>b</sup>	$1.70 \pm 0.08^{a}$
13	26.76	Cuminaldehyde	1257	24.85±2.81 <sup>b</sup>	29.20±1.51ª	28.25±0.46 <sup>a</sup>	28.89±1.30 <sup>a</sup>
14	28.63	2-Caren-10-al	1297	2.83±0.32 <sup>a</sup>	$2.99 \pm 0.19^{a}$	2.69±0.31 <sup>a</sup>	2.85±0.36 <sup>a</sup>
15	28.93	Cuminyl	1304	2.02±0.22°	3.05±0.33 <sup>b</sup>	$3.87 \pm 0.14^{a}$	$4.00 \pm 0.44^{a}$
16	29.31	Carvacrol	1313	$0.07 \pm 0.01^{a}$	$0.06 \pm 0.01^{a}$	$0.12 \pm 0.05^{a}$	$0.10 \pm 0.04^{a}$
17	30.36	$\rho$ -Mentha- 1 ,4- diene 7-al	1337	0.11±0.05 <sup>a</sup>	$0.08 \pm 0.01^{a}$	$0.09 \pm 0.09^{a}$	0.04±0.03 <sup>a</sup>
18	34.05	trans-Caryophylene	1422	$0.38 \pm 0.06^{a}$	0.30±0.05 <sup>a</sup>	0.42±0.00 <sup>a</sup>	$0.39 \pm 0.08^{a}$
19	34.21	cuminyl acetate	1434	$0.16 \pm 0.02^{a}$	0.09±0.01°	$0.12 \pm 0.05^{b}$	$0.12 \pm 0.02^{b}$
20	36.07	2-Thujene	1470	0.71±0.12 <sup>a</sup>	$0.55 \pm 0.08^{a}$	$0.74 \pm 0.01^{a}$	0.74±0.19 <sup>a</sup>
21	39.81	Elemicin	1563	2.87±0.52 <sup>a</sup>	2.72±0.57 <sup>a</sup>	3.27±0.14 <sup>a</sup>	3.13±0.58 <sup>a</sup>
22	40.75	Caryophyllene Oxide Butane, 1,2,3,4-	1587	0.58±0.11ª	0.57±0.14 <sup>a</sup>	0.74±0.06 <sup>a</sup>	0.68±0.17 <sup>a</sup>
23	43.82	tetrachlorohexafluoro	1668	1.00±0.19 <sup>a</sup>	$0.97 \pm 0.22^{a}$	1.25±0.12 <sup>a</sup>	1.15±0.30 <sup>a</sup>
24	44.49	$\alpha$ –Bisabolol	1686	$0.27 \pm 0.04^{a}$	0.19±0.01 <sup>a</sup>	0.30±0.05 <sup>a</sup>	0.27±0.17 <sup>a</sup>
		Total extraction time (Min)		240	240	240	240
		Yield (%) IC <sub>50</sub> (mg mL <sup>-1</sup> )		4.18 <sup>c</sup> 9.31 <sup>a</sup>	4.31 <sup>b</sup> 8.62 <sup>a</sup>	4.81 <sup>a</sup> 8.79 <sup>a</sup>	4.73 <sup>a</sup> 6.54 <sup>a</sup>

**Table 2.** The compositions and  $IC_{50}$  values of *B. persicum* essential oils obtained by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD) at three levels of microwave power (180, 360, and 540 W).

RT: Retention time, RI: Retention indices relative to  $C_9-C_{17}$  normal alkanes on the HP-5MS column.

\* Mean±Standard deviation (n= 2) based on the relative area percent of each chromatographic peak on a chromatogram.

<sup>\*\*\*</sup> In each row, means with different letters are significantly different (P<0.05).

Pourmortazavi *et al.* (2005) applied both supercritical fluid extraction and HD to isolate the essential oil from *B. persicum*. Higher levels of  $\gamma$ -terpinene and cuminaldehyde were found in the essential oil obtained by hydrodistillation.  $\rho$ -Cymene was not found in the essential oil extracted by supercritical fluid extraction and  $\alpha$ -Methyl-benzenemethanol was found only in the essential oil obtained by HD. In contrast, our results showed that the compositions of the essential oils by HD and MAHD were similar. Salehi *et al.* (2008) applied hydrodistillation-headspace solvent microextraction technique for the extraction and analysis of *B. persicum* essential oil and identified  $\gamma$ -terpinene, limonene, cuminaldehyde,  $\rho$ -mentha-1, 4-dien-7-al,  $\rho$ -

mentha-1, 3-dien-7-al and  $\rho$ -cymene as major components. This is in good agreement with the results of the present study. Other studies (Karim et al., 1977; Thappa et al., 1991; Baser et al., 1997) have also reported the composition of essential oil extracted with HD from B. persicum. In respect to the current study, Sadykov et al. (1978) reported higher levels of  $\rho$ -cymene (19.2%) and cuminaldehyde (40.7%). Azizi et al. (2009) identified the main components of essential oils from seeds of wild type B. persicum and compared them with those of seeds collected after 4 or 5 years of cultivation in comparison, our results showed lower amount of  $\gamma$ -terpinene (28.16-31.13%), but a much higher amount of cuminaldehyde (24.85-29.20%). The  $\alpha$ terpinene-7-al  $(\rho$ -mentha-1, 4-dien-7-al) content in our study was also much lower (0.04-0.11%). These results confirmed the reports based on the effects of environmental factors (soil, climate) and genetic variations (existence of chemotypes) on the quantity and quality of active substances of B. persicum essential oil (Azizi et al., 2009; Omidbaigi and Arvin, 2009).

# Antioxidant Activity: DPPH<sup>o</sup> Radical Scavenging Activity

The DPPH° assay has been widely used in recent years to estimate antioxidant activity of different compounds. Figure 3 shows the DPPH° scavenging activities of different concentrations of essential oil from B. persicum obtained through the various extraction methods applied in this study. The radical scavenging activities of the essential oils increased with an increase in their concentrations. Essential concentrations 50% oil providing inhibition  $(IC_{50})$  are shown in Table 2. The IC<sub>50</sub> values of the essential oils were found within the range of 6.45-9.31 mg ml<sup>-1</sup>, and were not significantly different among them. These findings are in agreement with the results of the antioxidant activity of rice bran oil extracted by microwaveassisted extraction (Zigoneanu *et al.*, 2008), where no significant differences were found among the DPPH<sup>o</sup> scavenging capacities of the extracts obtained by different solvents used in their study when microwave-assisted extraction and conventional solvent methods were used for the extraction of rice bran oil.

#### CONCLUSIONS

MAHD of the seeds of *B. persicum* was compared with the traditional HD. Essential oils obtained by MAHD and HD were very close in their compositions, but for the same yield, the time required for MAHD was much shorter than that for HD. No significant differences were found in the physical properties of essential oils obtained by MAHD and HD. DPPH<sup>o</sup> analysis of the extracted essential oils indicated that microwave irradiation did not adversely influence the antioxidant activity. Therefore, MAHD is an excellent alternative extraction method to obtain essential oils from *B. persicum*.

#### REFERENCES

- AACC. 1983. Approved Methods of the American Association of Cereal Chemists. 8<sup>th</sup> Edition, Library of Congress, Salem, MA.
- Ayoughi, F., Barzegar, M., Sahari, M. A. and Naghdibadi, H. 2011. Chemical Compositions of Essential Oils of *Artemisia dracunculus* L. and Endemic *Matricaria chamomilla* L. and an Evaluation of Their Antioxidative Effects. J. Agr. Sci. Tech., 3: 79-88.
- Azizi, M., Davareenejad, Gh., Bos, R., Woerdenbag, H. J. and Kayser, O. 2009. Essential Oil Content and Constituents of Black Zira (*Bunium persicum* [Boiss.] Burdenko Fedtsch.) from Iran during Field Cultivation (Domestication). J. Essent. Oil Res., 21: 78–82.
- Baser, K. H. C., Ozek, T., Abduganiv, B. E., Abdullaer, U. A. and Aripov, Kh. N. 1997. Composition of the Essential Oil of *Bunium*

persicum (Boiss.) B. Fedtsch. from Tajikistan. J. Essent. Oil Res., **9**: 597-598.

- Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of Free Radical Method to Evaluate Antioxidant Activity. *Zeitschrift Lebensmittel-Untersuchung Forschung*, 28: 25-30.
- 6. Eskilsson, C. S. and Bjorklund, E. 2000. Analytical-scale Microwave-assisted Extraction. J. Chromatogr. A, **902**: 227-250.
- Food Chemical Codex (FCC). 1996. 4<sup>th</sup> Edition, National Academic Press, Washington, DC, USA, 413 PP.
- Foroumadi, A., Asadipour, A., Amanzadeh, Y. and Arabpour, F. 2002. Composition of the Essential Oil of *Bunium persicum* (Boiss.) B. Fedtsch from Iran. *J. Essent. Oil Res.*, 14: 161-162.
- 9. Gholivand, M. B., Rahimi-Nasrabadi, M., Batooli, H. and Ebrahimabadi, A. H. 2010. Chemical Composition and Antioxidant Activities of the Essential Oil and Methanol Extracts of *Psammogeton canescens. Food Chem. Toxicol.*, **48**: 24–28.
- 10. Golmakani, M. T. and Rezaei, K. 2008a. Comparison of Microwave-assisted Hydrodistillation with the Traditional Hydrodistillation Method in the Extraction of Essential Oils from *Thymus vulgaris* L. *Food Chem.*, **109**: 925-930.
- Golmakani, M. T. and Rezaei, K. 2008b. Microwave-assisted Hydrodistillation of Essential Oils from *Zataria multiflora* Boiss. *Eur. J. Lipid Sci. Technol.*, **110**: 448-454.
- Iriti, B., M., Colnaghi, G., Chemat, F., Smadja, J., Faoro, F. and Visinoni F. A. 2006. Histocytochemistry and Scanning Electron Microscopy of Lavender Glandular Trichomes Following Conventional and Microwaveassisted Hydrodistillation of Essential Oils: A Comparative Study. *Flavour Fragr. J.*, 21: 704–712.
- Karim, A., Pervez, M. and Bhatty, M. K. 1977. Studies on The Essential of the Pakistan Species of the family Umbelliferae. Part 10. *Bunium persicum* Boiss. (Siah Zira) Seed Oil. *Pak. J. Sci. Ind. Res.*, 20: 106-108.
- Kaufmann, B. and Christen, P. 2002. Recent Extraction Techniques for Natural Products: Microwave-assisted Extraction and Pressurized Solvent Extraction. *Phytochem. Anal.*, 13: 105–113.
- 15. Omid Baigi, R. and Arvin, M. J. 2009. Effect of Growing Locations on the Essential Oil Content and Chemical Compositions of

Bunium persicum Boiss. Wild Growing in Iran. J. Essent. Oil-Bearing Plants, **12**: 34-40.

- Ondruschka, B. and Asghari, J. 2006. Microwave-assisted Extraction: A State of the Art Overview of Varieties. *Chimia*, 60: 321-325.
- Oroojalian, F., Kasra-Kermanshahi, R., Azizi, M. and Bassami, M. R. 2010. Phytochemical Composition of the Essential Oils from Three *Apiaceae* Species and Their Antibacterial Effects on Food-borne Pathogens. *Food Chem.*, **120**: 765–770.
- Özek, T., Özek, G., Baser, K. H. C. and Duran, A. 2005. Comparison of the Essential Oils of Three Endemic Turkish *Heracleum* Species Obtained by Different Isolation Techniques. *J. Essent. Oil Res.*, 17: 605–610.
- Ozkan, G., Sagdic, O., Gokturk, R. S., Unal, O. and Albayrak, S. 2010. Study on Chemical Composition and Biological Activities of Essential Oil and Extract from *Salvia pisidica*. *Food Sci, Technol.*, **43**: 186–190.
- 20. Pourmortazavi, S. M., Ghadiri, M. S. and Hajimirsadeghi, S. 2005. Supercritical Fluid Extraction of Volatile Components from *Bunium persicum* Boiss. (Black Cumin) and *Mespilus germanical*. (medlar) Seeds. J. Food Compos. Anal., **18**: 439-446.
- Rezvanpanah, S., Rezaei, K., Razavi, S. H. and Moini, S. 2008. Use of Microwave-assisted Hydrodistillation to Extract the Essential Oils from *Satureja hortensis* and *Satureja montana*. *Food Sci. Technol. Res.*, 14: 311 – 314.
- Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. 2005. Comparative Evaluation of 11 Essential Oils of Different Origin as Functional Antioxidants, Antiradicals and Antimicrobials in Foods. *Food Chem.*, **91**: 621–632.
- Sadykov, Y. D., Kurbanov, M., Khafizov, Kh. and Begovatov, Y. M. 1978. Composition of the Essential Oil from the Fruits of *Bunium persicum* (Boiss.) Burdenko Fedtsch. *Doklady Akademiya Nauk Republiki Tadzhikistan*, 21: 33-36.
- Salehi, P., Mohammadi, F. and Asghari, B. 2008. Seed Essential Oil Analysis of *Bunium persicum* by Hydrodistillation-headspace Solvent Microextraction. *Chem. Nat. Compou.*, 44(1): 111-113.
- Shahsavari, N., Barzegar, M., Sahari, M. A. and Naghdibadi, H. 2008. Antioxidant Activity and Chemical Characterization of Essential Oil of *Bunium persicum*. *Plant Foods Hum. Nutr.*, 63: 183–188.



- Stashenko, E. E., Jaramillo, B. E. and Martinez, J. R. 2004a. Analysis of Volatile Secondary Metabolites from Colombian *Xylopia aromatica* (Lamarck) by Different Extraction and Headspace Methods and Gas Chromatography. *J. Chromatogr. A*, 1025, 105–113.
- Thappa, R. K., Ghoshs Agarwal, S. G., Raina, A. K. and Jamwal, P. S. 1991. Comparative Studies on the Major Volatiles of Kala Zira (*Bunium persicum*) Seed of Wild and Cultivated Sources. *Food Chem.*, 41: 129-134.
- Wang, Z., Ding, L., Li, T., Zhou, X., Wang, L., Zhang, H., Ti Liu, L., Li, Y., Liu, Z., Wang,

H., Zeng, H. and He, H. 2006. Improved Solvent-free Microwave Extraction of Essential Oil from Dried *Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim. J. *Chromatogr. A*, **1102**: 11-17.

- 29. Wang, L. and Weller, L. C. 2006. Recent Advances in Extraction of Nutraceuticals from Plants. *Trends Food Sci. Technol.*, **17**: 300-312.
- Zigoneanu, I. G., Williams, L., Xu, Z. and Sabliov, C. M. 2008. Determination of Antioxidant Components in Rice Bran Oil Extracted by Microwave-assisted Method. *Bioresour. Technol.*, **99**: 4910-4918.

فعالیت آنتی اکسیدانی اسانس زیره سیاه ایرانی به دست آمده با روش تقطیر با آب به کمک مایکروویو

س. مزیدی، ک. رضایی، م. ت. گلمکانی، ا. شریفان، ش. رضازاده

# چکیدہ

برای استخراج اسانس از زیره ایرانی از روش های تقطیر با آب به کمک مایکروویو در سه توان (۸۸۰ ۲۹۶ و ۵۴۰ وات) و روش سنتی تقطیر با آب استفاده گردید. استخراج اسانس در روش تقطیر با آب به کمک مایکروویو در توان ۵۴۰ وات بسیار سریعتر از روش تقطیر با آب آغاز گردید (به ترتیب ۴ دقیقه در برابر ۳۸ دقیقه). وقتی که در روش تقطیر با آب، استخراج اسانس آغاز می شود، تقریباً ۵۰ درصد کل اسانس ۲/۱۵ درصد وزنی) در روش تقطیر با آب، استخراج اسانس آغاز می شود، تقریباً ۵۰ درصد کل اسانس ۲/۱۵ درصد وزنی) در روش تقطیر با آب به کمک مایکروویو در توان ۵۴۰ وات استخراج گردیده است. آنالیز (با استفاده از کروماتو گرافی گازی – طیف سنج جرمی) اسانس های به دست آمده با هر دو روش تقطیر با آب و تقطیر با آب به کمک مایکروویو نشان داد که ترکیبات اصلی تشکیل دهنده اسانس ها، گاما تریین (۲۱/۱۳–۲۸/۹ درصد وزنی وزنی)، کومین آلدئید (۲۰/۲–۲۰/۸ درصد)، پاراسیمن (۱۰/۹۰– ۱۴/۶۷ درصد) و لیمونن (۸۲/۸–۲/۱۴ درصد) بودند. در هر دو روش، ترکیبات اسانس مشابه بودند. فعالیت تریین (۱۳/۳–۲۸/۹ درصد) و لیمونن (۲۸/۵–۲/۱۴ درصد)، پاراسیمن (۱۰/۹۰– مایکا درصد) و لیمونن (۲۸/۵–۲/۱۴ درصد) بودند. در هر دو روش، ترکیبات اسانس مشابه بودند. فعالیت تریین (۲۰/۳–۲۰/۵ درصد) و درنی)، کومین آلدئید (۲۰/۲–۲۰/۵ درصد)، پاراسیمن (۱۰/۵۰– مایک انتی اکسیدانی بر اساس(IC<sub>5</sub>0) اسانس های استخراج شده به روش تقطیر با آب ۹/۱۰ میلی گرم در ترتیب ۱۴/۶۷ درصد) و فیمون زمان اسانس های استخراج شده به روش تقطیر با آب ۱۰/۹۱ میلی گرم در ترتیب ۱۶/۵۷ مراد و مرای روش تقطیر با آب به کمک مایکروویو در توان های ۱۸۰، ۳۰ و ۴۰۰ وات به ترتیب ۲۵/۵۰ مایک و مایی گرم در میلی لیتر بود. بر اساس نتایج این مطالعه، امواج مایکروویو اثر ترتیب ۱۵/۵۰ مایس و برای روش تقطیر با آب به کمک مایکروویو در توان های ۱۸۰، ۳۰ و ۲۵ دو تر ان ترتیب ۱۵/۵۰ موری و مایلی آسانس های استخراج شده ندارد و بنابر این می تواند به عنوان یک تامطلویی روی فعالیت آنتی اکسیدانی اسانس هری گرده.