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Analyses of the Essential Oil from *Bunium persicum* Fruit and its Antioxidant Constituents

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Abstract: This study was aimed to analyze and identify the antioxidant constituents of the essential oil of *Bunium persicum* (Apiaceae) fruit. The essential oil was obtained by hydrodistillation and analyses by GC-FID and GC-MS. The essential oil was tested for antioxidant capacity in DPPH radical scavenging and linoleic acid/β-carotene assays. The TLC-bioautography method based on DPPH radical assay and GC analyses were carried out to characterize the major antioxidant compounds in the essential oil. GC analyses showed the presence of sixteen compounds with *p*-cymene (31.1%), cuminaldehyde (22.2%), and γ-terpinene (11.4%) as the main components in the essential oil. The oil exhibited good radical scavenging [$IC_{50\ (DPPH^{\cdot})}$ = 4.47 (3.96 – 5.05) mg/mL] and antilipid peroxidation [$IC_{50\ (\beta\text{-carotene bleaching})}$ = 0.22 (0.16 – 0.31) mg/mL] activities. The TLC tests resulted in identification of cuminaldehyde, *p*-cymene-7-ol, and cuminyl acetate as the main constituents of the active oil fraction.

Key words: *Bunium persicum*, essential oil, chemical composition, antioxidant activity

1 INTRODUCTION

Antioxidants are considered as substances that can inhibit or retard oxidation processes. They protect cells from oxidative damage caused by the reactive oxygen species (ROS) and free radicals (FRs). The ROS and FRs cause extensive damage to cells leading to several chronic diseases such as diabetes, atherosclerosis, neurodegenerative diseases, etc. Therefore, dietary antioxidants have an important role in protecting the body from the active species. The oxidation mediated by ROS and FRs is also responsible for quality deterioration of unpreserved food and pharmaceutical products. Thus, synthetic antioxidants such as BHA and BHT are extensively used for industrial processing[1-4]. However, scientific investigations have reported that the high doses and/or long-term exposure to these compounds can cause toxic symptoms in animals so, their use is now restricted due to safety concerns[2, 5]. These findings have been amplified the attempts for the development of alternative antioxidants and much attention has been focused on the use of natural antioxidants in recent years.

Essential oils from plants and their constituents are well known to exert antioxidant activities thus; they are increasingly being studied as antioxidants. Generally, essential oils are widely used in the food, pharmaceutical and cosmetic industries as flavor agents. They also provide protection against oxidation and spoilage. For example, various plant essential oils (such as clove, oregano, rosemary, sage, and lavender) have been reported to exhibit strong antioxidant and lipid protection properties[6-9].

Bunium persicum (Boiss.) B. Fedtsch. [Syn. *Carum persicum* Boiss., *Carum heterophyllum* Regel & Schmalh.] is an aromatic plant belonging to the Apiaceae family. Its fruit has been widely consumed as a food flavoring agent and spice in Iran. Besides, the fruits have several therapeutic effects and they have been used for the treatment of colic, flatulence, indigestion, and dyspepsia[10, 11].

Some studies have been carried out on the chemical composition of the essential oil from *B. persicum* fruit. They indicated that the major compounds of the oil were γ-terpinene and cuminaldehyde[12-17]. Moreover, few studies showed that the essential oil from *B. persicum* fruit had antioxidant activity[18, 19]. However, to the best of our knowledge, the exact nature of the active constituents that contribute to the antioxidant activity of *B. persicum* essential oil is still unknown. The present study was investigated to analyze and identify the antioxidant components from *B. persicum* essential oil.

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2 EXPERIMENTAL

2.1 Plant material

B. persicum fruit was bought from a local market in Tehran, Iran in summer 2011 and authenticated by M. Kamalinejad at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences where the voucher specimens have been preserved.

2.2 Chemicals

All of the chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (France) and Merck Company (Germany).

2.3 Essential oil isolation

The fruit was crushed and subjected to the hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous sodium sulfate and stored under N_2 in a dark sealed vial at 4℃ until required.

2.4 Gas chromatography analyses

GC-FID analyses were carried out on an Agilent GC 7890A gas chromatograph equipped with a FID and a HP-5 capillary column (30 m × 0.25 mm, 0.25 μm film thicknesses). The initial oven temperature was held at 50℃ for 3 min, increased up to 120℃ with a heating rate of 3℃/min; then the column temperature was programmed as 120℃ to 250℃ by a heating rate of 5℃/min and held at this temperature for 5 min. The carrier gas was N_2 with a flow rate of 2 mL/min. The injector temperature and detector temperature were adjusted to 280℃ and 300℃, respectively. Sample size was 1.0 μL with a split ratio of 1:10.

GC-MS analyses were performed on an Agilent 7890A GC interfaced to an Agilent 7000 triple quad mass spectrometer. The operating conditions were the same conditions as described for GC-FID analyses, but the carrier gas was He. EI-MS spectra were recorded at 70 eV ionization voltage and the mass range was from *m/z* 50 to 1000 amu.

The identification of compounds was accomplished by comparing their mass spectra to those of the Wiley275.L library as well as their retention indices with those reported in the literature[20]. Retention indices were calculated using the retention times of *n*-alkanes (C_8 – C_{18}).

2.5 Antioxidant assays

2.5.1 DPPH˙ assay

The free radical scavenging capacity of *B. persicum* volatile oil was evaluated by a method based on the decolonization of DPPH radical [2,2'-Diphenyl-1-picrylhydrazyl][21]. Two mL of the DPPH˙ solution (40 μg/mL in methanol) was mixed with 200 μL of different dilutions of each sample in methanol, including positive controls (vitamin C and gallic acid), *B. persicum* oil, and its active compound (cuminaldehyde). The reaction mixture was allowed to stand at room temperature for 30 min then, the absorbance was recorded at 517 nm. The inhibition of DPPH˙ ($I_{DPPH^{\cdot}}$%) in percent was calculated by the following formula:

$$I_{DPPH^{\cdot}}(\%) = 100 \cdot \left[\frac{A_{control} - (A_{sample} - A_{blank})}{A_{control}}\right]$$

where A_{sample}, A_{blank}, and $A_{control}$ were the absorbance of sample, blank, and control, respectively.

2.5.2 Linoleic acid/β-carotene bleaching assay

The antilipid peroxidation activity of *B. persicum* essential oil was determined by the linoleic acid/β-carotene model[21]. A mixture of β-carotene and linoleic acid was prepared with 2 mL of a 200 μg/mL solution of β-carotene in chloroform, 45 μL of linoleic acid and 400 mg of Tween 40. Chloroform was evaporated under vacuum then, 100 mL of oxygenated distilled water was added to the residue. 0.5 mL of various dilutions of each sample in methanol, including positive controls (vitamin C and gallic acid) and *B. persicum* oil, and its active compound (cuminaldehyde), was added to 4.5 mL of the above mixture and the emulsion system was incubated in a hot water bath at 50℃ for 2 h. The initial absorbance at 470 nm (t = 0) for each reaction mixture was measured immediately. Subsequent absorbance values were obtained after incubation. The inhibition percentage of bleaching ($I_{bleaching}$%) was calculated using the following equation:

$$I_{bleaching}(\%) = (\text{Absorbance of sample after 2 h of assay / Initial absorbance of sample}) \times 100$$

2.5.3 Rapid screening for antioxidants

For screening of antioxidant compounds in *B. persicum* essential oil, the TLC-bioautography method was carried out[9, 22]. The diluted oil (1:10 in methanol) was spotted on silica gel sheets (silica gel 60 F_{254} TLC plates) and developed in *n*-hexane-ethyl acetate (9:1). Plates were sprayed with the methanolic solution of DPPH˙ (0.2%). The active constituents were detected as yellow spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

2.6 Activity guided fractionation of the essential oil for antioxidants

For the isolation and identification of the active compounds in the essential oil, PTLC was performed using the conditions previously described[9]. The regions showing DPPH˙ scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC-FID and GC-MS and also tested for their antioxidant activities.

2.7 Statistical analysis

All the experiments were carried out in triplicate. IC_{50} values were calculated from logarimic regression curves (*I*% against sample concentration) and presented with their respective 95% confidence limits. The one-way ANOVA followed by Tukey's post test was used for comparisons. A probability value of $p < 0.001$ was considered to denote a statistically significant difference. All the statistical analyses were accomplished using the computer software GraphPad Prism 3.02 for Windows (GraphPad Software, San Diego, CA, USA).

3 RESULTS AND DISCUSSION

The hydrodistillation of *B. persicum* fruit gave a colorless to yellowish oil with a yield of 2.2% (±0.1%) v/w. Sixteen compounds (representing of 91.8% of the total identified constituents) were identified in the oil by GC-MS analyses (Fig. 1). The identified compounds and their peak areas (%) have been given in Table 1. The oil consisted mainly of hydrocarbon monoterpens (55.3%) and oxygenated monoterpenoids (35.3%). The major compounds were *p*-cymene (31.1%), cuminaldehyde (22.2%), γ-terpinene (11.4%), and cuminyl acetate (9.4%). The above results revealed differences in composition with respect to data in

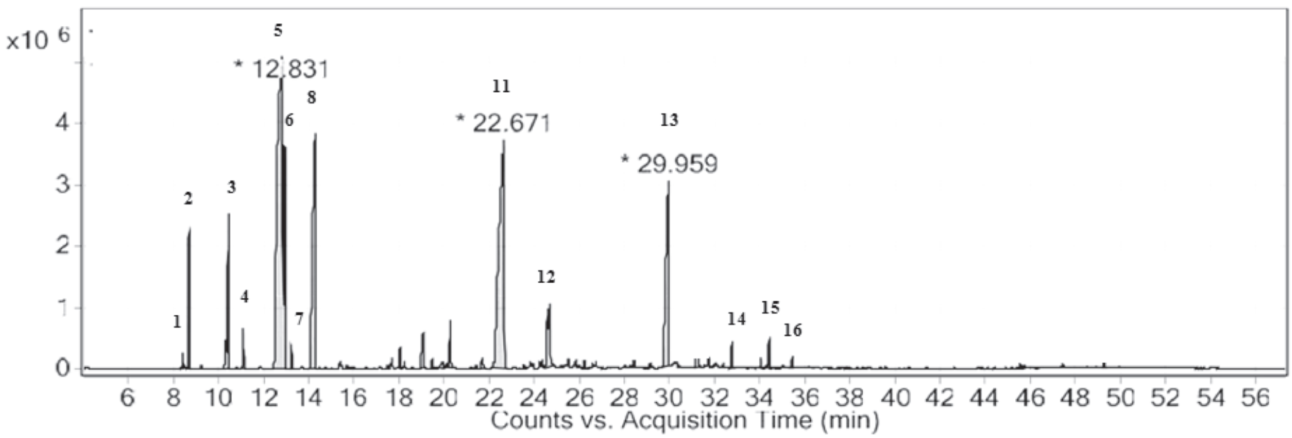

Fig. 1 GC-MS chromatogram of *Bunium persicum* essential oil. The identified compounds have been listed in Table 1.

Table 1 Chemical composition of *Bunium persicum* essential oil.

No.	Compound	RI	Peak area (%)
1	α-Thujene	925	0.3
2	α-Pinene	932	2.5
3	β-Pinene	975	4.1
4	β-Myrcene	989	0.8
5	*p*-Cymene	1029	31.1
6	Limonene	1031	4.7
7	(Z)- β- Ocimene	1038	0.4
8	γ- Terpinene	1061	11.4
9	Terpinene-4-ol	1172	0.2
10	*p*-Cymene-8-ol	1181	0.2
11	Cuminaldehyde	1243	22.2
12	*p*-Cymene-7-ol	1287	3.3
13	Cuminyl acetate	1423	9.4
14	Myristicin	1516	0.5
15	Caryophyllene oxide	1577	0.5
16	Dillapiol	1617	0.2
	Total identified constituents (%)		91.80

literature. Some other authors determined that the essential oil of *B. persicum* was characterized by the dominance of γ-terpinene and cuminaldehyde while, *p*-cymene and cuminaldehyde were found as the main constituents of the oil in this study. On the other hand, much higher concentration of cuminyl acetate was detected in the oil[12-17]. These differences in the composition of the essential oil of *B. persicum* could be attributed to several factors such as developmental stages, climatical and geographical conditions, existence of various chemotypes for the plant, etc[23].

The antioxidant capacity of the essential oil from *B. persicum* fruit was quantified with two different assays, including DPPH˙ and linoleic acid/β-carotene methods. Generally, the use of multiple measurements provides a better insight into the antioxidant potential of natural products. On the other hand, these two techniques are rapid and reliable methods to study the free radical scavenging and antioxidant activities of plant products[2,24].

The DPPH˙ decolonization test is widely employed to assess the antioxidant and free radical scavenging activities of natural sources. In this method, antioxidant molecules can quench DPPH radicals and convert them to colorless products[25]. *B. persicum* essential oil demonstrated a sigmoidal dose-response curve over the concentration range tested in the DPPH˙ assay (Fig. 2A). The IC_{50} value was 4.47 (3.96 – 5.05) mg/mL (Table 2).

The β-carotene bleaching method is a common test for determining of antioxidants to inhibit the lipid peroxidation in the propagation phase[26]. *B. persicum* oil showed a lipid peroxidation inhibitory activity in a concentration-dependent manner (Fig. 2B). The IC_{50} value was found to be 0.22 (0.16 – 0.31) mg/mL (Table 2).

Although, Shahsavari *et al.*[18] and Sharififar *et al.*[19] reported the essential oil of *B. persicum* had moderate antioxidant activities, the above findings showed that the oil was a potent antioxidant. Generally, essential oils are complex mixtures and consisted of many compounds. On the other hand, the antioxidant activities of the volatile oils are highly dependent on their chemical composition and content. Therefore, this complexity makes it usually difficult to characterize the activity pattern[27].

Because of significant antioxidant and free radical scavenging activities of *B. persicum* essential oil, it was further investigated to identify its active constituents. Therefore, a preliminary screening was initially carried out using DPPH˙ staining method on TLC. Application of *B. persicum* oil in the bioautography system mentioned above showed one main active band (F_1) with R_f value of 0.50 (Fig. 3).

As the essential oil presented a significant antioxidant activity in the assays and bioautography test, it was sub-

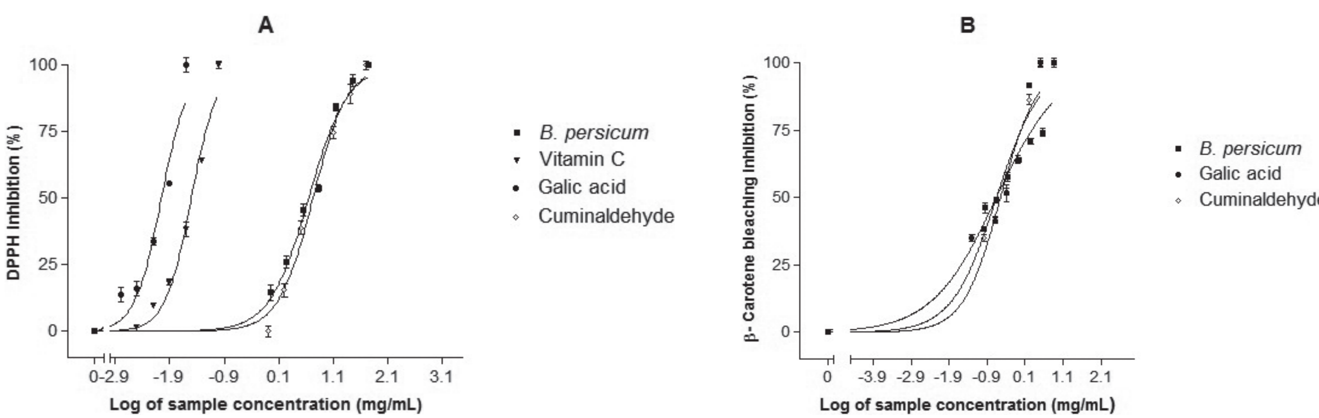

Fig. 2 Concentration-dependent free radical scavenging and antioxidant activities of *Bunium persicum* essential oil determined using (A) DPPH˙ method and (B) Linoleic acid/β-carotene bleaching technique. Each point represents the mean of three experiments.

Table 2 Free radical scavenging activity and antioxidant potency of *Bunium persicum* essential oil.

Sample	$IC_{50\ (DPPH^{\cdot})}$ (mg/mL)	$IC_{50\ (\beta\text{-carotene bleaching})}$ (mg/mL)
B. persicum essential oil	4.47 (3.96 – 5.05)^c	0.22 (0.16 – 0.31)^a
Cuminaldehyde	5.38 (4.79 – 6.04)^c	0.28 (0.23 – 0.34)^a
Gallic acid	0.009 (0.007 – 0.011)^a	0.22 (0.17 – 0.29)^a
Vitamin C	0.031 (0.028 – 0.035)^b	–

$^{(a\text{-}c)}$ Different superscript letters within each column denote significant differences ($p < 0.001$).

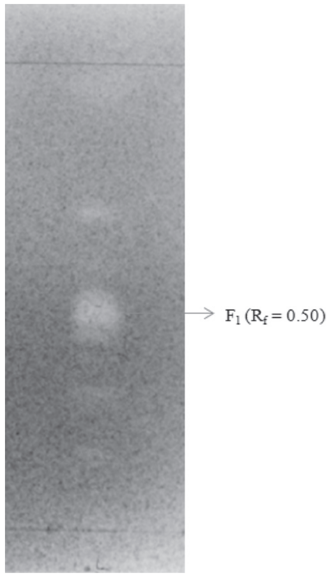

Fig. 3 TLC plate stained with 0.2% DPPH˙ solution.

jected to the PTLC for isolation of the active compounds. The active band was scratched out and eluted with chloroform and the compounds present in it were identified by GC-MS. Three components, including cuminaldehyde (55.7%), *p*-cymene-7-ol (12.0%), and cuminyl acetate (32.2%), were identified in this band (Fig. 4). Cuminaldehyde, as the major constituent of the active band, exhibited a favorable radical scavenging [$IC_{50 (DPPH^{\cdot})}$ = 5.38 (4.79 – 6.04) mg/mL] and lipid peroxidation inhibitory [$IC_{50 (\beta\text{-carotene bleaching})}$ = 0.28 (0.23 – 0.34)] activities.

4 CONCLUSION

The overall, *B. persicum* fruit essential oil studied here exhibited significant free radical scavenging and antioxidant activities in a series of *in vitro* tests. The bioautography screening of antioxidant compounds led to the identification of cuminaldehyde, *p*-cymene-7-ol, and cuminyl acetate as the major constituents of the active oil fraction.

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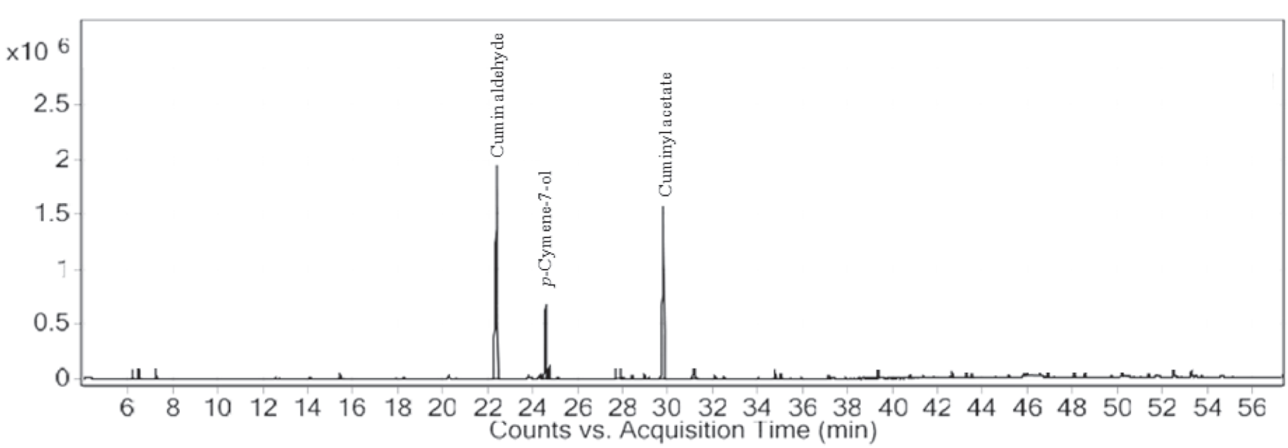

Fig. 4 GC-MS chromatogram of F_1 active band (R_f = 0.50) obtained from *Bunium persicum* essential oil.

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