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ORIGINAL PAPER

Antioxidant Activity and Chemical Characterization of Essential Oil of *Bunium persicum*

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Abstract The search for natural antioxidants, especially of plant origin, has notably increased in recent years. *Bunium persicum* Boiss. is an economically important medicinal plant growing wild in the dry temperature regions in Iran. In this study, chemical constituents of the essential oil of the seed from *Bunium persicum* Boiss. have been studied by GC/MS technique. The major components were caryophyllene (27.81%), γ-terpinene (15.19%), cuminyl acetate (14.67%). Individual antioxidant assays such as, DPPH$^{\bullet}$ scavenging activity and β-carotene bleaching have been carried out. In DPPH$^{\bullet}$ system, the EC_{50} value of essential oil was determined as 0.88 mg/mL. In β-carotene bleaching antioxidant activity of essential oil (0.45%) was almost equal to BHT at 0.01%. In addition, the antioxidant activity of the essential oil was evaluated in crude soybean oil by monitoring peroxide and thiobarbituric acid values of the oil substrate. The results showed that the *Bunium persicum* essential oil (BPEO) was able to reduce the oxidation rate of the soybean oil in the accelerated condition at 60 °C (oven test). The essential oil at 0.06% showed the same effect of BHA at 0.02%. Hence, BPEO could be used as an additive in food after screening.

Keywords Antioxidant activity · *Bunium persicum* Boiss · DPPH · Soybean oil

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Abbreviations

ANOVA	Analysis of variance
FID	Flame ionization detector
BCB	β-carotene bleaching
GC/MS	Gas chromatography–mass spectrometry
BHA	Butylated hydroxy anisole
LSD	Least significant difference
BHT	Butylated hydroxy toluene
PG	Propyl gallate
BPEO	*Bunium persicum* essential oil
PV	Peroxide value
DPPH$^{\bullet}$	2, 2′-diphenyl 1-picrylhydrazyl
TBA	Thiobarbituric acid

Introduction

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury and death [1]. Their deteriorative effects can be diminished by natural antioxidants available in foods. Also, oxidative reactions limit the shelf life of fresh and processed food stuffs and are a serious concern in food industry [2]. Antioxidants are substances that, when added to food products especially lipids and lipid-containing foods can increase shelf life of foods. Its mechanism is based on the retardation of lipid peroxidation, which is one of the major processes producing deterioration of food products during processing and storage [3]. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), and propyl gallate (PG) have been used as antioxidants since the beginning of the twentieth century. However, restrictions on the use of these compounds are being imposed because of their carcinogenicity [4]. Consequently, the need to identify alternative

natural and safe sources of food antioxidant arose [5] and the search for natural antioxidants, especially of plant origin, has notably increased in recent years [6–9]. Essential oils are known to poses multifunctional properties other than their classical roles as natural food additives. Besides the antibacterial, antifungal, and anti-inflammatory activities [10–12], many essential oils also have been confirmed to possess the antioxidant activity [13, 14].

Bunium persicum Boiss. is an economically important medicinal plant growing wild in the dry temperature regions in Iran. The seeds rich in essential oil, are consumed widely as condiment. In the indigenous system of medicines, seeds are regarded as stimulants and carminatives and found to be useful in diarrhea and dyspepsia [15]. In addition, this plant is used for culinary purposes and for flavoring foods and beverages [16].

The aims of this work were: (i) to determine the chemical composition of BPEO by using GC–MS, (ii) to evaluate the antioxidant activity by using the 2, 2′-diphenyl 1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging and β-carotene bleaching (BCB) methods, (iii) and to determine the antioxidant activity of BPEO in crude soybean oil by measuring of peroxide and thiobarbituric acid values (oven test).

Material and Methods

Materials

2, 2′-diphenyl 1-picrylhydrazyl (DPPH$^{\bullet}$), β-carotene, linoleic acid were purchased from Sigma Chemical Co. (MO, USA). Chloroform, ethyl acetate, carbon tetra chloride, acetic acid, potassium iodide, potassium iodate, starch, thiobarbituric acid, sodium thiosulfate, Tween 40, butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA) were purchased from Merck (Darmstadt, Germany). All chemicals were analytical grade. Crude soybean oil was purchased from Margarin factory in Varamin (Tehran, Iran). The plant material was obtained from a research farm of the Institute of Medicinal Plants and Natural Products Research, Karaj, Iran (56 °, 35′ N and 50 °, 58′ E, 1,500 m above sea level). Oils were extracted by hydrodistillation of the aerial parts using Clevenger-type apparatus for 3 h [17]. The oils were dried over anhydrous sodium sulphate and kept at −4 °C until it was used. The essential oil yield was about 2.2%.

GC/MS Analysis of Essential Oil

GC analysis was carried out on an Agilent Technologies 6890 gas chromatograph equipped with flame ionization detector (FID) and a HP-5 capillary column (30 m× 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 50 °C for 5 min, and then programmed at 3 °C min^{-1} to 240 °C and after that programmed at 15 °C min^{-1} to 300 °C (held for 3 min) and finally reached 340 °C (at 3 °C min^{-1}). Other operating conditions were: carrier gas, He with a flow rate of 0.8 mL/min; injector and detector temperatures were 290 °C and 209 °C, respectively; split ratio, 1:10. GC/MS analysis was performed on a GC mentioned above coupled with a Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions as described above, mass spectra were taken at 70 eV. Mass range was from m/z 35–375 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature [18, 19] and presented in the MS computer library.

Antioxidant Activity

DPPH$^{\bullet}$ Assay

The hydrogen atom or electron-donation ability of the BPEO was measured from the bleaching of the purple-colored ethyl acetate solution of DPPH$^{\bullet}$. This spectrophotometric assay uses stable 2, 2′-diphenyl 1-picrylhydrazyl radical (DPPH$^{\bullet}$) as a reagent. Two mL of various concentrations of the samples (0.045–0.45% *w*/*v*) in ethyl acetate were added to 1 mL of a 2×10^{-4} M solution of DPPH$^{\bullet}$. The decrease in absorbance at 517 nm was determined by Scinco spectrophotometer (Seoul, South Korea) after 1 h for all samples. Ethyl acetate was used as blank. The absorbance of the ethyl acetate solution DPPH$^{\bullet}$ radical without antioxidant was measured as control. All determinations were performed in triplicate and results were averaged. The percentage inhibition of the DPPH$^{\bullet}$ radical by the sample was calculated according to the following formula [20]:

$$\%\ \text{Inhibition} = \left(\left(A_{C(0)} - A_{S(t)}\right)/A_{C(0)}\right) \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t=0 min and $A_{S(t)}$ is the absorbance of the sample at t. Essential oil concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting percentage of remaining DPPH$^{\bullet}$ against BPEO concentration.

β-Carotene Bleaching Assay

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. A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.2 mg of

β-carotene was dissolved in 10 mL of chloroform and 1 mL was added to 20 mg linoleic acid and 200 mg of Tween 40. Chloroform was gently removed under a stream of nitrogen gas. Then, 50 mL of distilled water, saturated with oxygen (30 min, 100 mL min^{-1}), was added with vigorous shaking. 200 μl of ethanolic stock solution of sample and BHT were separately mixed with 5 mL emulsion. Reading of all samples were taken immediately at t=0 min by spectrophotometer at 470 nm. The cuvettes were incubated in a water bath at 50 °C for 30 min. Then, absorbance of samples at 470 nm was determined by spectrophotometer [21]. All determinations were performed in triplicate and results were averaged. The percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \left(A_{\text{sample}(t)} - A_{\text{control}(t)}\right) / \left(A_{\text{control}(0)} - A_{\text{control}(t)}\right) \times 100$$

where $A_{\text{sample}(t)}$ and $A_{\text{control}(t)}$ are the absorbance of the sample and control at t, respectively, and $A_{\text{control}(0)}$ is absorbance of the control at t=0 min

Effect of BPEO on Soybean Oil Oxidation

The BPEO added to crude soybean oil at 0.02%, 0.04% and 0.06%. Synthetic antioxidants (BHA, BHT), at 0.01%, 0.02% were used for comparison. The oven test method at 60 °C was used to check stability. Oxidation was periodically assessed by the measurement of peroxide value (PV) at 0th, 8th, 16th, 24th and 32th days of storage according to the AOCS method [22], and thiobarbituric acid (TBA) value of the samples at the same days of storage according to the method described by Sidewell et al. [23]. A control sample was prepared under the same conditions, without adding any antioxidant. All determinations were performed in triplicate and results were averaged.

Statistical Analysis

Data were analyzed statistically using analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.01$ using least significant differences (LSD) test (by SAS software). The data are presented as mean ± standard deviation of the three determinations.

Results and Discussion

Chemical Composition of the BPEO

The results obtained by GC–MS analysis of the BPEO of the plant are presented in Table 1. Twenty-nine compounds were identified, representing 98.25% of the total essential oil.

Table 1 Chemical composition of BPEO (%) determined by GC/MS

Components	Kovat's retention index	Retention time (min)	Composition (%)
α-Thujene	928	16.83	0.27
α-Pinene	936	17.29	1.51
Sabinene	976	19.49	0.28
β-Pinene	980	19.75	2.23
Myrecene	991	20.33	0.65
α-Terpinene	1,019	21.85	0.23
P-Cymene	1,030	22.40	5.25
Limonene	1,034	22.64	3.62
1, 8-Cineol	1,039	22.89	0.40
γ-Terpinene	1,063	24.43	15.19
Terpinolene	1,094	25.84	3.49
Linalool	1,102	26.22	0.14
Terpinene-4-ol	1,184	30.42	0.40
P-Cymen-8-ol	1,199	30.76	0.19
Cuminaldehyde	1,245	33.71	5.96
Thymol	1,296	35.75	1.90
α-Methyl-benzene methanol	1,302	35.98	3.88
Pinocarvyl acetate	1,320	37.79	4.47
Geranyl acetate	1,386	39.78	0.47
Cuminyl acetate	1,428	41.98	14.67
γ-Elemene	1,440	42.39	0.20
Caryophyllene	1,487	44.08	27.81
α-Selinene	1,501	44.94	0.70
β-Bisabolene	1,511	45.43	0.22
Croweacin	1,525	46.15	2.89
Spathulenol	1,581	48.56	0.11
Caryophyllene oxide	1,597	48.84	0.25
Dillapiole	1,628	50.06	0.31
α-Bisabolol	1,691	52.38	0.40
Total			98.25

Caryophyllene (27.81%), γ-terpinene (15.19%), cuminyl acetate (14.67%), cuminaldehyde (5.96%), p-cymene (5.25%), pinocarvyl acetate (4.36%), limonene (3.88%), α-methyl-benzene methanol (3.88%), croweacin (2.89%) and β-pinene (2.23%), were the main components of BPEO.

It would also be noteworthy to point out that the composition of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions [24]. Literature is limited about the chemical composition of the *Bunium persicum* essential oil. Abduganiew et al. analyzed the BPEO originating from Tajikistan, and they detected 22 compounds, including p-mentha-1, 4-dien-7-al, γ-terpinene, β-pinene and cumin aldehyde [15].

DPPH$^{\bullet}$ Assay

In our study, antioxidant activity of BPEO was studied as *in vitro* and in a food system. The model of scavenging the

stable DPPH• radical is a widely used method to evaluate antioxidant activity in a relatively short time compared the other methods should be pointed out [25]. Antioxidant activity of essential oil tested by the DPPH• model system and the antioxidant activity of BPEO was found as IC_{50}=0.88 mg mL^{-1}. BHT used as a synthetic antioxidant in food industry showed higher antioxidant activity (IC_{50}= 0.58 mg mL^{-1}, [20]) when it was compared to essential oil. The addition of essential oil to the DPPH• solution caused a rapid decrease in the absorption at 517 nm. The degrees of discoloration indicate the radical scavenging capacity of the essential oil. Free radicals cause autoxidation of unsaturated lipids in food [26]. The effect of antioxidant on DPPH• radical scavenging was thought to be due to their hydrogen donating ability [27].

The radical scavenging effect of the studied essential oil (by DPPH• method) increased with increasing its concentration. In comparison with BHT and α-tocopherol, essential oil has been found less effective than these synthetic antioxidants. IC_{50} values of the *Bunium persicum* essential oil has been compared with other essential oils and BHT and α-tocopherol in Table 2. As seen from Table 2, radical scavenging power of BPEO is higher than activities reported in other papers [20, 28–32]).

β-Carotene Bleaching Assay

The β-carotene bleaching (BCB) method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. This fact is used in the antioxidant activity evaluation of the BPEO in comparison with BHT. Figure 1 shows the antioxidant activity of the BPEO in comparison with BHT. Antioxidant activity of the BPEO at 0.45% *v/w* was similar to BHT at

Table 2 Comparison of DPPH radical scavenging capacity (IC_{50} value) of BPEO with some natural and synthetic antioxidants

Sample	IC_{50}	Reference
BPEO	0.88±0.04 (mg ml^{-1})	This work
Petroselinum crispum	80.21±3.41 (mg ml^{-1})	[20]
Origanum vulgare SSP. *vulgare*	8,900±0.5 (μgml^{-1})	[28]
Marrubium globosu subsp. *globosum*	1,203±7.18 (μgml^{-1})	[32]
Thymus vulgaris L.	8.9 (mg ml^{-1})[a]	[31]
Satureja hortensis L.	5.8 (mg ml^{-1})[a]	[31]
Cyclotrichium origanifolium.	17,100±0.5 (μgml^{-1})	[30]
Thymus sipyleus subsp. *sipyleus* var. *sipyleus*	2,670±0.5 (μgml^{-1})	[29]
BHT	0.58±0.02 (mg ml^{-1})	[20]
α-tocopherol	0.10±0.00 (mg ml^{-1})	[20]

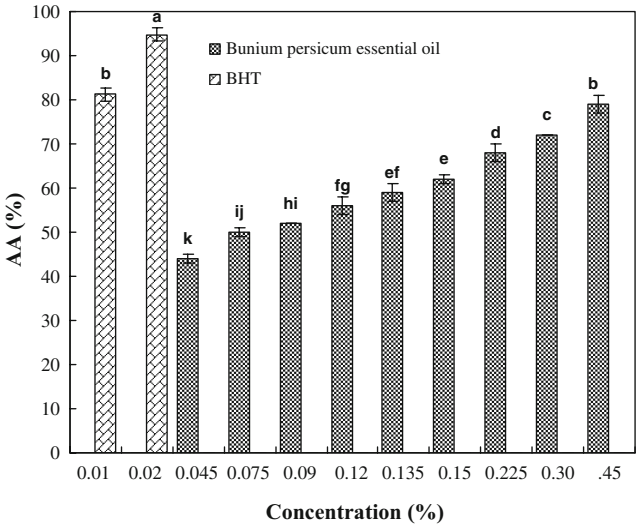

Fig. 1 Antioxidant activity of BPEO and BHT at different concentrations determined by β-carotene bleaching method

0.01% ($P \leq 0.01\%$). The antioxidant activity of the essential oil increased with increasing concentration. In the literature, there are many reports indicating the antioxidant potential of the γ-terpinene [13, 33]. According to their report, monoterpenes hydrocarbons present antioxidant activity due to the presence of strongly activated methylene groups, and this is clearer in β-carotene–linoleic acid system where

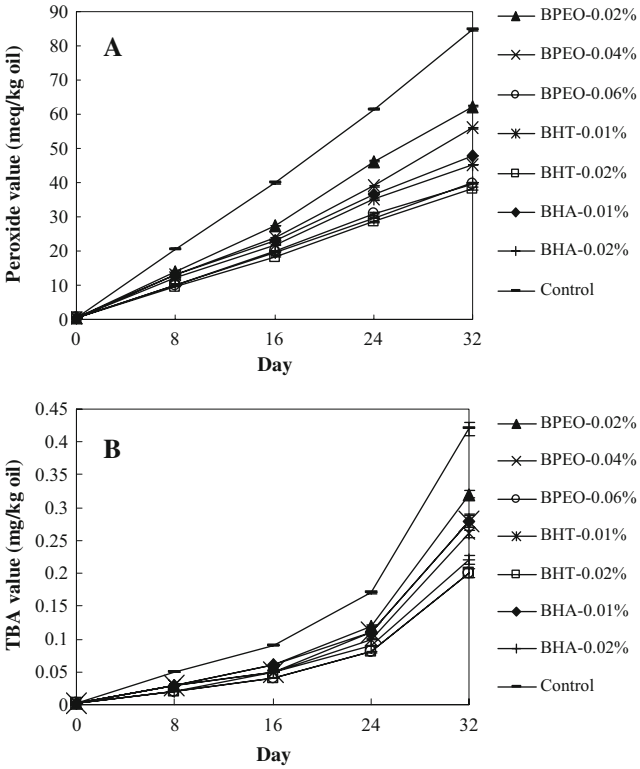

Fig. 2 Effect of BPEO on soybean oil oxidation expressed as peroxide (**A**) and thiobarbituric acid (**B**) values during storage at 60 °C

a competition with the activated methylene in C-11 of linoleic acid may be hypothesized. This can explain the better activity of γ-terpinene in linoleic acid oxidation system [13].

Effect of BPEO on Soybean Oil Oxidation

The addition of natural and synthetic antioxidant to soybean oil affected, to different degree, the peroxide and TBA values during accelerated oxidation at 60 °C for 32 days (Fig. 2) of storage. PV measures primary products of lipid oxidation, and TBA measures the formation of secondary oxidation products, mainly malonaldehyde, which may contribute off-flavours to oxidized oil [34]. All samples with BPEO level added at 0.02–0.06% were more stable on heating at 60 °C than the control, when assessed by the change in peroxide (Fig. 2a) and TBA (Fig. 2b) values. The antioxidant effect of BPEO increased with concentration and, at a concentration of 0.06%, the antioxidant activity was not significantly different ($P<0.01$) from that of the BHA at 0.02%. The results proved a stabilization effect of BPEO in the crude soybean oil. These findings are consistent with the results of Farag et al. who found that extracts of thyme and clove at 1,200 and 2,400 ppm, respectively, produced an antioxidant power similar to that produced by BHT at 200 ppm [35]. A study of the oregano antioxidant activity in mackerel oil showed that the effect of 0.5% oregano was comparable to that of 0.02% BHA [36]. Rehman et al. compared the antioxidant activity of potato peel extract in soybean oil with synthetic antioxidants (BHA and BHT) and reported that there was no distinct difference between synthetic antioxidants (200 ppm) and potato peel extract (1,600 ppm) in inhibition of soy bean oil peroxidation [37]. Unlike synthetic antioxidants, BPEO can be added in larger quantities to get optimal effects (addition of synthetic antioxidants is limited under food laws and regulations). Therefore, lipid peroxidation can be controlled by the required amount of BPEO.

In this study, by using three different methods, confirmed that the BPEO posses antioxidant properties. However, further investigation and the antioxidant activity mechanism are warranted. These studies can be useful as a starting point for further application of BPEO in food preparations.

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