Genet Resour Crop Evol (2014) 61:1621–1631
DOI 10.1007/s10722-014-0158-6

NOTES ON NEGLECTED AND UNDERUTILIZED CROPS

Bunium persicum: variability in essential oil and antioxidants activity of fruits from different Iranian wild populations

Remigius Chizzola · Amir Hossein Saeidnejad · Majid Azizi · Fatemeh Oroojalian · Hossein Mardani

Received: 27 December 2013 / Accepted: 22 July 2014 / Published online: 27 August 2014

Abstract The fruits of *Bunium persicum* (Boiss.) B. Fedtsch were collected throughout Iran from ten populations and assessed for their essential oil composition and antioxidant activity. The volatiles were analyzed by GC/MS/FID after microdistillation and SPME. All 10 accessions had the same major volatiles roughly in the order γ-terpinene = cuminal > γ-terpinen-7-al > *p*-cymene > limonene > α-terpinen-7-al although they origin from quite different sites. Nevertheless these plants that are able to grow in a wide range of environments may present the base for domestication with the aim to optimize essential oil yield with a high proportion of aldehydes. The methanolic extracts of the fruits showed a moderate antioxidant potential.

Keywords Apiaceae · *Bunium persicum* · Cuminaldehyde · Essential oil · α-Terpinen-7-al · γ-Terpinen-7-al

R. Chizzola (✉)
Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria
e-mail: Remigius.Chizzola@vetmeduni.ac.at

A. H. Saeidnejad
Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

M. Azizi
Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

F. Oroojalian
Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran

H. Mardani
Department of International Environmental and Agricultural Science, Tokyo University of Agriculture and Technology, Tokyo, Japan

Introduction

Since ancient times, herbs and spices have been added to food to improve the flavor and organoleptic properties, but also as preservatives. In recent years, the essential oils and the herbal extracts from various species of edible and medicinal plants have attracted a great deal of scientific interest due to their potential as a source of natural agents to increase the safety and shelf life of foods and of natural biologically active compounds (Bozin et al. 2006). Essential oils are very complex natural mixtures which can contain about 20–60 components at quite different concentrations.

Bunium persicum (Boiss.) B. Fedtsch or black cumin is a plant from the Apiaceae family, especially grown in different regions of Iran. *B. persicum* seeds are called "zireh kuhi", meaning "wild cumin", and are used as a culinary spice (Mortazavi et al. 2010). In the indigenous system of medicines, seeds are regarded as stimulants and carminatives and found to

Fig. 1 Fruit of *Bunium persicum*

be useful in diarrhea and dyspepsia (Baser et al. 1997). Also, this plant is used for culinary purposes and for flavoring foods and beverages. Additionally anti-fungal properties as activity against *Fusarium* species may present a further promising application of this plant (Sekine et al. 2007). The considerable point is the plant has been forced into the endangered category due to the recent relentless extraction of seeds obtained from wild habitats. The main depletion factor has been found to be the thoughtless and unscientific commercial collection of its seeds for rapid financial gains. The competition for its seeds is so severe that, instead of collecting the ripe seed, the entire plant is removed even when the seeds are immature (Azizi et al. 2009). Overexploitation is not only a matter of concern in Iran but also in the Himalaya (Sofi et al. 2009).

Bunium persicum might have originated in the area between Central Asia and Northern India. It is found growing naturally in alpine and sub-alpine habitats of northwestern Himalayas at an altitude of 1,800–3,500 m. It generally grows in forests, grassy slopes, and to some extent in low alpine pastoral lands (Sofi et al. 2009). It is a temperate plant, economically important, naturally occurring in the dry temperature and elevated regions where the winter is severe and the ground is under snow in winter, because a long chilling period is essential for germination of seeds (Saeidnejad et al. 2013a). These regions include mainly a wide geographical distribution in Iran and also some other areas such as Afghanistan, Pakistan, Tajikistan and North India (Hanelt et al. 2001; Panwar 2000; Panwar et al. 1993).

Plant varies from dwarf (30 cm) to tall (80 cm) compact or spreading, moderately to highly branched, tuberous and perennial herb (Panwar 2000). The stem is often hollow in the internodal region with secretory canals containing essential oils and resins. The leaves are freely, 2–3 pinnate with finely dissected and filiform sections. The flowers are small, white in color with readily symmetrical small sepals, petals and stamens. The bracts are linear, sometimes divided, and bracteoles are absent with asymmetrical rays (Sofi et al. 2009). The mericarps are oblong to oblong-lanceolate with three longitudinal ridges, a small stylopodium and styles <1 mm (Fig. 1).

The chromosome number is $2n = 14$. The plant has not been subjected to any vigorous crop improvement work and there are no approved varieties or improved cultivars (Panwar 2000). A recent study regarding to the genetic diversity of Iranian *B. persicum* germplasm by morphological markers showed that the heritability of morphological traits was high and the ecotypes exhibited a high genetic variations from the viewpoint of coefficient of variations (Azimzadeh et al. 2012). Despite knowing high economical value and high quality genetic sources of *B. persicum*, there are lots of unknown and unstudied cases about this amazing species.

It was reported that *B. persicum* seeds are rich in essential oil (up to 7 %), with a higher amount of monoterpene aldehydes than other monoterpenes (Thappa et al. 1991). Generally, the main components are cuminaldehyde, *p*-mentha-1,3 dien-7-al (=α-terpinene-7-al) and *p*-mentha-1,4-dien-7-al (=γ-terpinene-7-al); terpene hydrocarbons are γ-terpinene, *p*-cymene, β-pinene and limonene. The latter compounds are thought to reduce the quality of the spice (Thappa et al. 1991; Baser et al. 1997; Foroumadi et al. 2002).

Azizi et al. (2009) studied the content and constituents of two wild population of *Bunium persicum* which were cultivated under farm conditions and found γ-terpinene as the main constituent, which was associated with cuminaldehyde and γ-terpinen-7-al. Pourmortazavi et al. (2005) analyzed volatile constituents in a supercritical fluid extract of *B. persicum* using gas chromatography–mass spectrometry and identified a total of 16 compounds. γ-terpinene (38 %), cuminaldehyde (11 %) and α-methyl benzenemethanol (26 %) were the major compounds. Although there are some information about the chemical composition of the essential oil of *B. persicum,* there is no information about the chemical

Fig. 2 Geographical distribution of the collected ecotypes on different provinces of Iran. Name and climatic properties of each region is presented on Table 1

composition of different ecotypes from different parts of Iran. Thus, the aim of this study is to investigate the essential oil content and constituents of ten ecotypes of *B. persicum* collected from different regions of Iran.

Materials and methods

Plant materials and samples collection

In order to find the differences between different ecotypes of *B. persicum* the main habitats of the plant were located and climatic properties of each region were evaluated. Afterward, ten areas were selected and ten ecotypes were collected from natural habitats located in seven provinces of Iran (Kerman, Yazd, Qazvin, Semnan, Bandar-abbas, Fars and Khorasan Razavi) (Fig. 2). Climatic information and soil samples of each area were also collected (Table 1). Fruits were hand threshed to minimize any damage to the fruits. A round holes sieve (2 mm diameter) was used to clean fruit lots. Fruits were also separated manually from other particles with similar size and empty or half-filled seed. They were stored at 4–6 °C prior to chemical analysis.

Analysis of the volatile fraction

Hydrodistillation

About 15 g from the whole seeds were subjected with 200 mL distilled water to hydrodistillation for 3 h in a Clevenger type apparatus. The resulting essential oil was collected and stored at −18 °C until further analysis.

Microdistillation

As only small sample amounts were available from some accessions the distillation was carried out using the automatic microdistillation unit MicroDistiller from Eppendorf (Hamburg, Germany). About 0.2–0.3 g finely crushed dried plant material and 10 mL distilled water were filled into the sample vial. The collecting vial, which contained 1 mL water, 0.5 g NaCl and 300 µL *n*-hexane was connected with a capillary to the sample vial. The heating program applied to the sample vial was 15 min at 108 °C and then 45 min at 112 °C. The collecting vial was kept at −2 °C, where the volatiles were trapped in 0.3 mL *n*-Hexane containing 0.06 mg hexadecane as internal standard. Each sample was microdistilled twice.

Table 1 Origins of the accession, annual precipitation, altitude and soil properties of habitats of the evaluated accession of *Bunium persicum*

Accession	Collected location	Altitude (m)	Annual precipitation (mm)	Soil properties were plants grown			
				Texture	EC (dS/m)	pH	Organic carbon (%)
1	Zar mountain (Damghan, Semnan province)	1,650	77	Clay	0.6	7.8	1.7
2	Alamoot mountain (Qazvin province)	1,500	300–400	Loamy sand	0.4	7.6	1.6
3	Geno mountain (Bandar-Abbas, Hormozgan province)	2,050	80–120	Clay	0.6	7.6	1.6
4	Khajeh forest (Kelat, Khorasan Razavi province)	920	200–250	Clay	0.5	7.8	1.7
5	Chelmir (Daregaz, Khorasan Razavi province)	260	1,300	Loamy sand	0.5	7.3	1.4
6	Lakhse mountain (Mehriz, Yazd province)	1,040	195	Loam	0.7	7.6	2.2
7	Freezi village (Chenaran, Khorasan Razavi province)	1,680	230	Clay	0.6	7.5	1.9
8	Toodaj mountain (Estahban, Fars province)	1,860	340	Silty loam	0.6	7.5	1.9
9	Margiri mountain (Joopar, Kerman province)	2,950	155	Loam	0.7	7.6	2.2
10	Mashhad (Khorasan Razavi province)	979	241	Silty loam	0.6	7.4	1.8

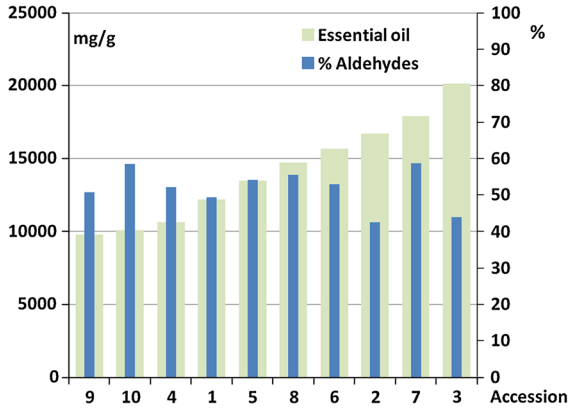

Fig. 3 Essential oil content and proportion of the aldehyds (cuminal + γ-terpinen-7-al + α-terpinen-7-al in the accessions of *Bunium persicum*

SPME

20 mg of the seeds were cut with a razor blade to open the oil ducts and put into a 10 mL vial together with a filter paper disc (6 mm in diameter) soaked with 10 µL of camphor as internal standard (0.1 mg/mL in methanol). The vial was tightly closed with a septum and further processed in the CTC-PAL autosampler with the mounting for SPME fibers.

So the SPME fiber (PDMS-DVB Polydimethylsiloxane-Divinylbenzene, Supelco, Bellefonte, PA, USA) was exposed for 30 min at 50 °C while stirring to the headspace of the sample. Afterwards the fiber was introduced into the injection port of the GC system and desorbed for 3 min at 250 °C. Each sample was analyzed twice with SPME.

GC and GC–MS

The analyses were carried out on an Agilent Technologies 7,890 A gas chromatograph equipped with a 5,975 C quadrupole mass selective detector, a flame ionization detector (FID) and a CTC-PAL autosampler. The separation was done on a 30 m × 0.25 mm fused silica column coated with 0.25 µm HP5-MS with helium as carrier gas at a constant flow rate of 1.4 mL/min. The injector temperature was held at 250 °C. The compounds eluting from the column were distributed with a Deans switch at equal proportions to the detector of the mass spectrometer (MSD) and FID. The total ion current (*m/z* 40–400) from the MSD was used to identify the compounds according to their mass spectra and their retention indices (McLafferty 1989; Adams 2007). The FID signal without any correction was used to calculate percentage compositions or the amounts of the essential oil constituents. In the latter case the same response as for the internal standard was assumed.

The temperature program consisted of three different temperature levels. Temperature was held 1 min at 50 °C, followed by 5 °C/min up to 220 °C, finally rose to 280 °C with 15 °C/min. For the SPME analyses the split ratio was set at 10:1. The injection volume of liquid samples, generated by microdistillation or

hydrodistillation, was 1 µL with a split ratio of 100:1. The FID was operated at 250 °C and supplied with 30 mL/min H_2 and 300 mL/min air.

Polyphenols and antioxidant activity

Extraction: About 50 mg of the finely powdered fruits were extracted with 8 mL methanol for 30 min in an ultrasonic bath. The extracts were filtered and kept at −18 °C until further analysis. The measurements of polyphenols and antioxidative activity were based on colorimetric reactions and were adapted to be measured with a microplate reader (Bio Rad, iMark).

Total phenolics: This fraction were assayed with the Folin-Ciocalteu reagent. In the wells of the microplate 10 µL extracts was added to 100 µL aqua dest. followed by 5 µL Folin-Ciocalteu reagent, 10 µL Na_2CO_3 (35 % in aqua dest.) and again 125 µL aqua dest. After 1 h resting in the dark the plate is measured at 750 nm. To calibrate the color formation seven concentration steps (ranging from 0.2 to 2.5 µg caffeic acid in 110 µL were taken instead the sample and the initial water volume.

Total flavonoids: The assay is based on the reaction of flavonoids with $NaNO_2$ and $AlCl_3$ which gives pink colored complexes (Leontowicz et al. 2003). The total sample volume was 140 µL (40 µL extract and 100 µL aqu. dest). To this portion 15 µL $NaNO_2$ (2.5 % in aqu. dest.) was added followed by 15 µL aluminium chloride (10 % $AlCl_3 \cdot 6\ H_2O$ in aqu. dest). After 5 min there was an addition of 50 µL 1 n NaOH and further 5 min later the plate was measured at 490 nm. The calibration curve was constructed with increasing concentration of catechine (1–10 µg in 140 µL instead of the sample).

DPPH-test: Antioxidants react with the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) which is decolorized. A portion of the sample (5–25 µL) made up to 100 µL methanol were incubated for 30 min in the dark with 100 µL DPPH reagent (0.015 % in methanol) in the microplate wells. Increasing volumes (0–8 µl) of trolox (0.62 mg/mL in ethanol) made up to 100 µL with methanol instead of the samples were used to obtain a calibration curve. A preparation consisting of 50 µL trolox, 50 µL aqu. dest. and 100 µL DPPH reagent where the DPPH was completely decolorized was taken as blank and subtracted from all measurements. The decolorisation was measured at 490 nm.

FRAP: Antioxidants are able to reduce ferric (Fe^{+++}) ions. The resulting ferrous ions (Fe^{++}) form a deep blue complex with 2,4,6-tripyridyl-*s*-triazine (TPTZ) (Benzie and Strain 1996). In the microplate wells 9 µL sample, 15 µL aqua dest. and 180 µL working reagent were mixed and measured after 5 min at 595 nm. The working reagent consisted of 25 mL acetic acid buffer (300 mmol/L), pH 3.6, 2.5 mL 10 mmol/L TPTZ in 40 mmol/L HCl and 2.5 mL $FeCl_3$ solution (20 mmol/L). A calibration curve was generated using increasing amounts of trolox from 0.06 to 2.4 µg/well (7 steps) instead of the samples.

Statistical analysis

The statistical analyses were done with the package SPSS for Windows, version 17.0. A hierarchical cluster analysis using the Euclidian distance was carried out to group the *B. persicum* accessions according to their essential oil composition. The percentages of 17 compounds of the microdistillates have been taken into consideration. Pearson correlation coefficients were calculated to address relationships between soil parameters, essential oils and antioxidant tests.

Results and discussion

The volatiles in the fruits of *B. persicum* were analyzed with different methods. Accessions 4 and 10 where enough fruits were available were extracted by a classical hydrodistillation. They yielded 2.3 and 2.4 % (v/w) essential oil whose composition is shown in Table 2. Both oils were similar with γ-terpinene as main compound followed by γ-terpinen-7-al and cuminaldehyde. Together the aldehydes (cuminal, γ-terpin-7-al and α-terpinen-7-al) represented 47.3 and 47.6 % of the essential oil fraction.

The fruits from all 10 accessions were extracted by microdistillation and the resulting oil fractions analyzed by GC (Table 2). This technique is a good alternative to a classical hydrodistillation and gives usually comparable results (Baser et al. 2006; Kurkcuoglu et al. 2003). Cuminaldehyde was the

Table 2 Composition of the essential oils from the hydrodistillation of two selected *Bunium persicum* accessions (2 and 4) and mean composition of the volatiles obtained by microdistillation (MD) and SPME

	RI	Hydrodistillation				MD		SPME	
		4 %	10 %	4 µg/g	10 µg/g	Mean %	SD	Mean %	SD
α-Thujene	930	0.3	0.2	55	46	0.2	<0.1	0.2	<0.1
α-Pinene	937	0.6	0.6	134	168	0.9	0.6	1.2	0.6
Camphene	953	0.1		16		<0.1	0.1	0.1	0.1
Sabinene	977	0.7	0.5	143	147	0.7	0.1	0.8	0.3
β-Pinene	980	1.3	1.4	266	388	1.6	0.9	2.2	1.4
Myrcene	992	0.6	0.4	126	98	0.5	0.1	0.7	0.1
δ-3-Carene	1,013					<0.1	<0.1	0.1	<0.1
α-Terpinene	1,020	0.1		12		0.1	<0.1	0.1	<0.1
p-Cymene	1,028	14.2	9.9	2,941	2,767	13.5	2.2	11.0	1.4
Limonene	1,033	6.2	7.3	1,272	2,029	5.8	2.9	5.6	2.8
1,8-Cineole	1,039					0.3	0.2		
γ-Terpinene	1,064	26.3	30.7	5,446	8,586	23.0	3.6	32.0	3.1
cis-Sabinene hydrate	1,071	0.1		30		0.2	<0.1	0.2	<0.1
Terpinolene	1,091	0.3	0.3	63	77	0.4	0.1	0.4	0.2
trans-Sabinene hydrate	1,100					0.1	<0.1	0.1	<0.1
Terpinen-4-ol	1,182	0.5	0.3	96	93	0.5	0.1	0.2	<0.1
p-Cymene-8-ol	1,189					<0.1	<0.1	0.1	<0.1
α-Terpineol	1,194	0.1		12		0.1	<0.1	0.1	0.1
Caranone	1,197	0.5	0.3	166	191	1.0	0.2	0.4	0.1
Cumin aldehyde	1,250	19.8	17.3	4,100	4,831	27.8	4.7	27.2	2.5
p-Menth-1-en-7-al	1,281	0.1	0.1	19	34	<0.1	<0.1	0.1	<0.1
α-Terpinen-7-al	1,290	5.2	4.7	1,085	1,310	3.8	0.9	2.1	0.5
γ-Terpinen-7-al	1,298	22.3	25.6	4,632	7,152	19.2	5.1	12.4	2.2
β-Bourbonene	1,392							<0.1	<0.1
β-Elemene	1,397							<0.1	<0.1
β-Caryophyllene	1,429							0.4	0.2
trans-α-Bergamotene	1,442							<0.1	<0.1
E-β-Farnesene	1,459							<0.1	<0.1
α-Humulene	1,464							<0.1	<0.1
γ-Muurolene	1,484							<0.1	<0.1
ar-Curcumene	1,487							0.1	<0.1
Germacrene D	1,490							0.2	0.1
α-Zingiberene	1,499							0.2	0.1
E,E-α-Farnesene	1,513							0.3	0.1
β-Sesquiphellandrene	1,530							0.1	0.1
cis-α-Bisabolene	1,547							<0.1	<0.1
Caryophyllene oxide	1,594							<0.1	<0.1
α-Bisabolol	1,692							<0.1	<0.1

RI retention index, *SD* standard deviation
n = 10

dominating monoterpene followed by γ-terpinene, γ-terpinen-7-al and *p*-cymene. Limonene, α-terpinen-7-al and β-pinene were minor compounds (about 1.5–6 %) whereas all other compounds afforded <1 % of the oil. Although the samples were similar, a classification was attempted based on the percentages of the oil compounds (Fig. 4). Accession 10 had the highest γ-terpinen-7-al content (32 %), accession 9

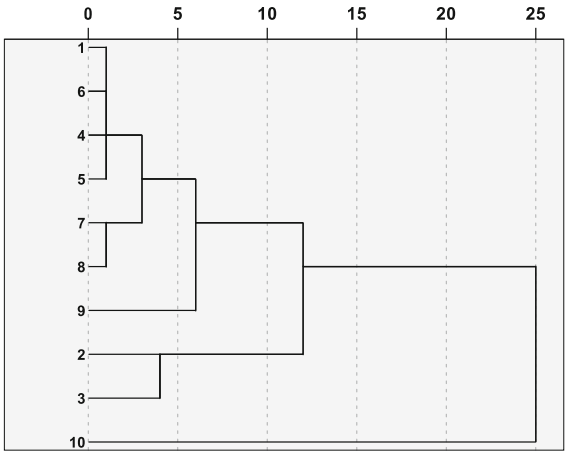

Fig. 4 Classification of the accessions (1–10) based on essential oil compounds obtained in microdistillation, calculated with percentages

the highest limonene content (11.8 %). In accessions 2 and 3 the contents were γ-terpinene > cuminal > γ-terpinen-7-al, while in accessions 7 and 8 the ranking was cuminal > γ-terpinen-7-al > γ-terpinene and in accessions 1, 4, 5 and 6 cuminal > γ-terpinene > γ-terpinen-7-al. so the highest proportions of aldehydes were found in the accessions 7, 10 and 8 (Fig. 3).

The microdistillation unit does not allow to read directly the content of distilled oil, but it can be calculated approximately by applying the response of internal standard hexadecane to the sum of the peaks in the chromatogram. This is displayed in Fig. 3 and shows that between the accessions the oil contents can roughly vary from 10 to 20 mg/g with the accessions 3, 7 and 2 having the highest oil contents. In the similar way the content of the individual compounds was calculated and summarized in Table 3. There it can be seen that for the main compounds cumin aldehyde, γ-terpinene and γ-terpinen-7-al mean contents of 3,982, 3,322 and 2,734 μg/g were calculated, respectively. For cuminaldehyde, γ-terpinene and *p*-cymene, the highest content recorded was about three times higher than in the accession with the lowest content. And accessions 9, 10 and 4 were those with the lowest and accessions 7, 3 and 2 those with the highest contents (Fig. 3).

The volatiles of *B. persicum* fruits were also analyzed with SPME coupled to GC. SPME has become in the recent years a widely applied technique to characterize volatiles in a wide range of matrices. It is a solvent free technique that relies on the repartition of the volatiles between the sample matrix, the headspace and the fiber. The mean composition of the volatile fraction adsorbed on the fiber is also presented in Table 2. The main compounds found were γ-terpinene (mean 32.0 %) and cuminaldehyde (mean 27.2 %) followed by γ-terpinen-7-al, *p*-cymene, limonene, β-pinene and α-terpinen-7-al. These were the same main compounds as already found in the microdistillates, but in the SPME analysis a higher γ-terpinene to γ-terpinen-7-al ratio appeared in comparison to the microdistillates. The SPME method is very sensitive and can detect small amounts of sesquiterpenes as <0.3 % of the total peak area each, compounds that were not present in the distillates or microdistillates (Table 2). Similarly in *Thapsia* species sesquiterpenes as γ-cadinene and guaienes were considerably better extracted by SPME than recovered in distilled oils (Drew et al. 2012). Camphor was added as internal standard to the samples and the amount of volatiles extracted with the fiber was approximately calculated assuming the same response as camphor. The calculated contents were lower than those calculated in the microdistillates and the span between lowest and highest contents was for the main compounds usually <2. The reason for this is in the distinct adsorption of the individual compounds to the SPME-fiber and the circumstance that different internal standards for microdistillation and SPME have been used. Furthermore it is necessary to use low amounts of sample to not overload the fiber and a inhomogeneous repartition of the volatiles between individual fruits may lead to higher standard deviations in repeated analyses (Krüger 2007). Indeed the comparison of SPME volatiles with a classical distilled essential oil has been assessed differently ranging from similar to very different. In *Chaerophyllum aromaticum* L. fruits SPME gave a higher proportion of germacrene D as compared to an essential oil obtained by hydrodistillation while β-pinene was higher in the distillate (Chizzola 2009). The volatiles from German chamomile flower heads as isolated by SPME were dominated by *E*-β-farnesene, artemisia ketone and germacrene D while the distilled oil differed clearly with α-bisabolol oxide A and chamazulene as main oil compounds (Rafieiolhossaini et al. 2012).

The essential oil composition of *B. persicum* has already been reported by several authors. Their results are summarized in Table 5. All oils contained *p*-

Table 3 Variability of the volatile compounds in the 10 accessions as determined in the microdistillates (MD) and by SPME (μg/g)

	Mean	Median	Min	Max	Order of accessions*
Cumin aldehyde					
MD	3,982.0	4,195.5	2,089.5	5,986.4	10,9,4,1,2,3,5,8,6,7
SPME	2,850.1	2,750.2	2,350.7	3,512.8	1,3,9,10,5,4,2,6,8,7
γ-Terpinene					
MD	3,321.7	3,011.1	1,970.9	5,962.7	9,10,4,8,1,5,7,6,2,3
SPME	3,386.8	3,150.4	2,630.4	4,416.0	9,1,3,5,8,6,10,4,7,2
γ-Terpinen-7-al					
MD	2,733.8	2,661.0	1,779.7	3,933.8	9,4,1,5,2,6,8,10,7,3
SPME	1,301.8	1,287.2	901.4	1,621.2	9,5,4,10,6,3,8,2,1,7
p-Cymene					
MD	1,922.9	1,857.4	945.6	2,891.5	10,9,4,5,1,8,6,7,2,3
SPME	1,171.3	1,060.0	858.4	1,574.3	10,5,3,9,6,1,8,4,7,2
Limonene					
MD	783.4	720.8	360.1	1,414.6	4,5,10,8,6,7,1,3,9,2
SPME	587.1	570.1	287.4	1,181.4	3,8,10,5,6,1,7,4,2,9
α-Terpinen-7-al					
MD	541.4	551.5	349.7	694.5	9,4,1,2,8,5,10,6,7,3
SPME	215.0	203.2	173.7	305.5	9,8,7,1,2,6,5,3,4,10
β-Pinene					
MD	213.1	173.1	86.0	508.0	5,10,6,7,9,1,2,3,4,8
SPME	223.8	174.5	62.4	480.2	2,6,1,5,10,7,9,4,3,8
Caranone					
MD	138.9	128.4	75.7	255.7	4,10,9,1,8,7,2,5,6,3
SPME	41.7	41.3	24.6	62.9	1,10,4,9,8,3,7,5,2,6
α-Pinene					
MD	117.5	90.0	52.9	293.8	5,10,6,7,9,2,1,3,4,8
SPME	126.1	104.8	39.0	257.0	6,1,5,10,7,2,9,4,3,8
Sabinene					
MD	94.4	93.7	58.0	157.7	9,10,4,1,5,7,8,6,2,3
SPME	85.8	73.7	57.2	204.6	1,5,9,6,3,10,8,7,4,2
Myrcene					
MD	78.0	68.8	47.2	170.4	10,9,5,1,4,8,6,7,2,3
SPME	71.5	68.3	55.2	106.8	1,6,5,9,8,3,10,2,7,4
Terpinen-4-ol					
MD	73.1	69.5	48.6	97.2	9,10,4,1,8,5,3,7,6,2
SPME	20.7	20.8	16.4	27.7	5,10,9,8,1,3,4,6,7,2
Terpinolene					
MD	50.9	42.5	27.8	81.6	3,6,10,7,2,8,9,5,4,1
SPME	44.7	45.0	13.2	77.5	3,6,8,1,4,5,2,7,9,10
1,8-Cineol					
MD	43.4	46.1	0.0	82.6	9,2,1,10,4,5,8,6,7,3
SPME	–	–	–	–	
α-Thujene					
MD	35.3	35.5	20.3	61.8	10,9,4,1,8,5,7,6,2,3

Table 3 continued

	Mean	Median	Min	Max	Order of accessions*
SPME	23.0	22.2	18.0	29.5	1,5,3,9,6,10,8,2,4,7
cis-Sabinene hydrate					
MD	21.8	20.9	14.7	34.0	9,10,1,4,8,5,7,6,3,2
SPME	17.0	16.6	12.8	24.1	5,1,9,3,8,6,10,4,7,2

* From lowest to highest content

Table 4 Phenolics and antioxidant activity in methanolic extracts of the fruits

Accession	Total phenolics (mg/g)	Total flavon (mg/g)	DPPH (mg/g)	Fe-reduction (mg/g)
1	12.8	4.3	10.4	6.4
2	9.0	4.5	9.5	6.1
3	12.0	5.6	11.2	7.5
4	8.9	4.5	8.0	5.7
5	12.5	5.1	9.7	6.9
6	9.6	4.4	9.2	6.2
7	8.7	4.9	10.7	7.2
8	10.5	6.1	10.6	7.7
9	14.7	9.5	14.8	10.9
10	8.9	6.4	10.7	7.0

cymene, γ-terpinene and cumin aldehyde, some of them also γ-terpinen-7-al and α-terpinen-7-al, all compounds that occurred in major proportions also in the samples of the present study. Cuminylalcohol and phenylpropanoids as myristicin and elemicin, reported occasionally in literature, were not present in the actual study. An earlier report worked out that amongst the main compounds seeds of cultivated plants contained more cuminaldehyde (27.3–34.1 %), α-terpinen-7-al and γ-terpinen-7-al (29.6–36.8 %), while in wild collected seeds there were more γ-terpinene (25.6–42.9 %) and *p*-cymene (24.0–27.8 %) and less aldehydes (Thappa et al. 1991). Another working group found that seeds from two Iranian sites contained more cuminaldehyde than those from two other sites (Omidbaigi and Arvin 2009). A further geographical differentiation in the oil composition has been observed where the major component in three Iranian, one Pakistan and one Indian populations were γ-terpinene (39.7–41.9 %), α-terpinen-7-al (37.2 %) and cuminaldehyde (37.1 %), respectively (Jahansooz et al. 2012). Also the influence of environmental stress has been studied. Plants exposed to drought stress had

Table 5 Main compounds (%) in the essential oils from *Bunium persicum* as described in literature

Reference	A	B	C	D	E	F	G	H
Provenance	Tadjikistan	Iran	Iran	Iran	Iran	Iran	East. Iran	Iran
Plant part	Fruits	Fruits	Fruits	Fruits	Fruits	Aerial parts	Fruits	Fruits
p-Cymene	5.3	8.0	10.6	12.1	5.6	5.3	13.3	6.7
Limonene	0.0	2.0	6.3	5.1	10.6	3.6	7.6	5.9
γ-Terpinene	25.7	44.2	35.5	25.8	45.7	15.2	31.1	46.1
Cumin aldehyde	11.7	16.9	18.9	27.0	12.7	6.0	24.9	15.5
α-Terpenen-7al	5.1	0.4	2.4					
γ-Terpinen7-al	29.0	10.5	6.0				0.1	0.1
Further compounds	β-Pinene 15.6	1,8-Cineol 2.9	β-Pinene 2.6	β-Pinene 3.1	Cuminyl alcohol 6.4	Cuminyl acetate 14.7	β-Pinene 3.3	Cuminyl alcohol 7.4
				Cuminyl alcohol 6.0		Caryophyllene 27.8	Elemicin 2.9	
				Myristicin 2.5				

A: Baser et al. (1997), B: Azizi et al. (2009), Oroojalian et al. (2010), C: Mortazavi et al. (2010), D: Foroumadi et al. (2002), E: Pourmortazavi et al. (2005), F: Shahsavari et al. (2008), G: Mazidi et al. (2012), H: Sharififar et al. (2010)

lower fruit yields but higher oil contents in the fruits while the essential oil composition changed little (Saeidnejad et al. 2013b).

Finally Talei and Mosavi (2009) reported an Iranian hydrodistilled *Bunium persicum* seed oil having a completely different composition containing isopulegyl acetate (24.6 %) anethol (20.4 %) and camphor (10.4 %) as main compounds, which were completely absent in the present accessions. This oil was devoid of the aldehydes.

In the present study there were found some differences between the 10 accessions but all had the same major volatiles roughly in the order γ-terpinene = cuminal > γ-terpinene-7-al > *p*-cymene > limonene > α-terpinene-7-al although they origin from quite different sites ranging from 260 to 2,950 m altitude with 155–1,300 mm annual precipitation but growing all on slightly alkaline soils (pH 7.3–7.8) as displayed in Table 1. An analysis correlating soil parameters, calculated essential yield from microdistillation and percentages of individual oil components showed a weak negative trend between altitude and precipitation ($r = -0.619$, $p = 0.056$) but no correlation between any of the soil parameters and essential oil yield or composition. Amongst the individual oil compounds, *p*-cymene was negatively correlated with α-terpinen-7-al ($r = -0.78$, $p = 0.008$) and γ-terpinen-7-al ($r = -0.89$, $p = 0.001$) while α-terpinen7-al was positively correlated with γ-terpinen-7-al ($r = 0.86$, $p = 0.001$).

Finally, total phenolics, total flavonoids and antioxidant activity in methanolic extracts from the fruits has been measured as displayed in Table 4. Some variability was found. Total phenolics ranged from 8.7 to 14.7 mg caffeic acid equivalents per gram plant material, and total flavonoids from 4.3 to 9.5 mg/g catechin equivalents. Antioxidant activity varied from 9.2 to 14.4 mg Trolox equivalents in the DPPH test and from 6.1 to 10.9 mg/g in the Fe-reduction test. In the correlation analysis total phenolics and total flavonoids were well correlated with DPPH ($r = 0.69$, $p = 0.026$ and $r = 0.90$, $p < 0.001$, respectively) and Fe-reduction ($r = 0.68$, $p = 0.029$ and $r = 0.96$, $p < 0.001$). Additionally DPPH was correlated with Fe-reduction ($r = 0.96$, $r < 0.001$). Also a correlation between altitude and total flavonoids ($r = 0.66$, $p = 0.038$), DPPH ($r = 0.81$, $p = 0.005$) and Fe-reduction($r = 0.76$, $p = 0.011$), suggesting that the higher altitudes constitute an environmental stress situation leading to a higher production of antioxidants.

Some antioxidant activity in *Bunium persicum* has also been demonstrated by different authors. The essential oil showed good antioxidant potential in five different test systems and gave 50.7 mg gallic acid equivalents/mL (Zangiabadi et al. 2012). Nickavar and Abolhasani (2009) reported a total flavonoid content of 20.2 mg/g as rutin-equivalents in an $AlCl_3$ based colour reaction different from that used in the present study. Sharififar et al. (2010) pointed out γ-terpinene and kaempferol as major antioxidant compounds in *B. persicum*. However, comparing obtained activities with literature results difficult because of the multitude of test systems, the different standard antioxidants used and the different sample preparation. In general, the essential oil shows higher activity than solvent extracts from the fruits (Sharififar et al. 2010). Altogether *B. persicum* fruits appear to display low to medium antioxidant potential (Nickavar and Abolhasani 2009).

Conclusion

Bunium persicum is able to grow in a wide range of environments and presented the same major essential oil compounds in slightly varying proportions. The tested accessions may serve as basis for the domestication of this plant with the aim to optimize essential oil yield with a high proportion of aldehydes. Both methods microdistillation and SPME are suitable to obtain volatile fingerprints for the characterization of *B. persicum* fruits.

Acknowledgments The authors wish to thank Mr. Heinz Pulletz for technical assistance during the analyses of the volatile oils.

References

Adams RP (2007) Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, 4th edn. Allured, Carol Stream

Azimzadeh M, Amiri R, Assareh MH, Bihamta MR, Forootan M (2012) Genetic diversity of Iranian *Bunium persicum* germplasm by morphological markers and essential oil components. J Med Plants Res 6(7):1119–1129

Azizi M, Davareenejad G, Bos R, Woerdenbag HJ, Kayser O (2009) Essential oil content and constituents of black zira

(*Bunium persicum* [Boiss.] Fedtsch.) from Iran during field cultivation (domestication). J Essen Oil Res 21:78–82

Baser KHC, Özek T, Abduganiev BE, Abdullaev UA, Aripov KN (1997) Composition of the essential oil of *Bunium persicum* (Boiss.) B.Fedtsch. from Tajikistan. J Essen Oil Res 9:597–598

Baser KHC, Özek G, Özek T (2006) Composition of the essential oil of *Chaerophyllum macropodum* Boiss. fruits obtained by microdistillation. J Essen Oil Res 18:515–517

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Analyt Biochem 239:70–76

Bozin B, Mimica-Dukic N, Simin N, Anackov G (2006) Characterization of the volatile composition of essential oil of some Lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54:1822–1828

Chizzola R (2009) Composition of the essential oil of *Chaerophyllum aromaticum* (Apiaceae) growing wild in Austria. Nat Prod Commun 4:1235–1238

Drew DP, Rasmussen SK, Avato P, Simonsen HT (2012) A comparison of headspace solid-phase microextraction and classic hydrodistillation for the identification of volatile constituents from *Thapsia* spp. provides insight into guaianolide biosynthesis in Apiaceae. Phytochem Anal 23:44–51

Foroumadi A, Asadipour A, Arabpour F, Amanzadeh Y (2002) Composition of the essential oil of *Bunium persicum* (Boiss.) B. Fedtsch. from Iran. J Essen Oil Res 14:161–162

Hanelt P, Institute of Plant Genetics and Crop Plant Research (IPK) (eds) (2001) Mansfeld's encyclopedia of agricultural and horticultural crops. Springer, Berlin

Jahansooz F, Sefidkon F, Najafi A, Ebrahimzadeh H, Najafi MS (2012) Comparison of essential oils of *Bunium persicum* (Boiss.) populations grown in Iran, Pakistan and India. J Essen Oil Bear Plants 15:761–765

Krüger H, Schulz H (2007) Analytical techniques for medicinal and aromatic plants. StewartPostharv Rev 4(4):1–12

Kurkcuoglu M, Sargin N, Baser KHC (2003) Composition of volatiles obtained from spices by microdistillation. Chem Nat Comp 39:355–357

Leontowicz M, Gorinstein S, Leontowicz H, Krzeminski R, Lojek A, Katrich E, Ciz M, Martin-Belloso O, Soliva-Fortuny R, Haruenkit R, Trakhtenberg S (2003) Apple and pear peel and pulp and their influence on plasma lipids and antioxidant potentials in rats fed cholesterol-containig diets. J Agric Food Chem 51:5780–5785

Mazidi S, Rezaei K, Golmakani MT, Sharifan A, Rezazadeh S (2012) Antioxidant activity of essential oil from Black Zira (*Bunium persicum* Boiss.) obtained by microwave-assisted hydrodistillation. J Agric Sci Tech 14:1013–1022

McLafferty FW (1989) Registry of mass spectral data, 5th edn. Wiley, New York

Mortazavi SV, Eikani MH, Mirzaei H, Jafari M, Golmohammad F (2010) Extraction of essential oils from *Bunium persicum* Boiss. using superheated water. Food Bioprod Process 88:222–226

Nickavar B, Abolhasani FA (2009) Screening of antioxidant properties of seven Umbelliferae fruits from Iran. Pak J Pharm Sci 22:30–35

Omidbaigi R, Arvin MJ (2009) Effect of growing locations on the essential oil content and chemical compositions of *Bunium persicum* Boiss. wild growing in Iran. J Essen Oil Bear Plants 12(1):34–40

Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami MR (2010) Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. Food Chem 120:765–770

Panwar K (2000) Black caraway. In: Arya PS (ed) Spice crops of India. Kalyani Publishers, New Delhi, pp 172–178

Panwar K, Agarwal J, Sharma S (1993) Economic viability of Kala zeera cultivation in high altitude dry temperate regions of Himachal Pradesh. Agric Situat India 48: 151–154

Pourmortazavi SM, Ghadiri M, Hajimirsadeghi SS (2005) Supercritical fluid extraction of volatile components from *Bunium persicum* Boiss. (black cumin) and *Mespilus germanica* L. (medlar) seeds. J Food Compos Anal 18: 439–446

Rafieiolhossaini M, Adams A, Sodaizadeh H, van Damme P, de Kimpe N (2012) Fast quality assessment of German chamomile (*Matricaria chamomilla* L.) by headspace solid-phase microextraction: influence of flower development stage. Nat Prod Commun 7:97–100

Saeidnejad AH, Khajeh-Hosseini M, Askarzadeh MA (2013a) Breaking dormancy of seeds from eight populations of *Bunium persicum* (Apiaceae). Seed Sci Technol 41(3):452–457

Saeidnejad H, Kafi M, Khazaei HR, Pessarakli M (2013b) Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*. Turk J Bot 37:930–939

Sekine T, Sugano M, Majid A, Fujii Y (2007) Antifungal effects of volatile compounds from black zira (*Bunium persicum*) and other spices and herbs. J Chem Ecol 33:2123–2132

Shahsavari N, Barzegar M, Sahari MA, Naghdibadi H (2008) Antioxidant activity and chemical characterization of essential oil of *Bunium persicum*. Plant Foods Hum Nutr 63:183–188

Sharififar F, Yassa N, Mozaffarian V (2010) Bioactivity of major components from the seeds of *Bunium persicum* (Boiss.) FEDTCH. Pak J Pharm Sci 23:300–304

Sofi PA, Zeerak NA, Singh P (2009) Kala zeera (Bunium persicum Bioss.): A Kashmirian high value crop. Turk J Biol 33:249–258

Souri E, Amin G, Farsam H, Barazandeh Tehrani M (2008) Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. DARU J Pharm Sci 16:83–87

Talei GR, Mosavi Z (2009) Chemical composition and antibacterial activity of *Bunium persicum* from west of Iran. Asian J Chem 21:4749–4754

Thappa RK, Ghosh S, Agarwal SG, Raina AK, Jamwal PS (1991) Comparative studies on the major volatiles of Kalazira (*Bunium persicum* seed) of wild and cultivated sources. Food Chem 41:129–134

Zangiabadi M, Sahari M, Barzegar M, Naghdi Badi H (2012) *Zataria multiflora* and *Bunium persicum* essential oils as two natural antioxidants. J Med Plants 11(41):8–21