CHEMIJA. 2010. Vol. 21. No. 1. P. 59–62

Menthone- and estragole-rich essential oil of cultivated *Ocimum basilicum* L. from Northwest Iran

Mohammad Bagher Hassanpouraghdam[1]*,

Abbas Hassani[2],

Mohammad Safi Shalamzari[3]

[1] *Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran*

[2] *Department of Horticultural Sciences, Faculty of Agriculture, Urmian University, Urmia 5715944931, Iran*

[3] *Research Service Laboratory, Faculty of Chemistry, University of Tabriz, Tabriz, Tabriz 51666, Iran*

The hydrodistilled essential oil from aerial parts of cultivated *Ocimum basilicum* L. plants from Northwest Iran was analyzed by gas chromatography / mass spectrometry. Forty seven components were identified, comprising 97.9% of total oil. Monoterpenoids (77.8%) prevailed among the essential oil components, followed by the lesser share of sesquiterpenoids (12.8%). Oxygenated monoterpenes (75.3%) were the predominant components of oil with menthone (33.1%), estragol (21.5%), isoneomenthol (7.5%), menthol (6.1%) and pulegone (3.7%) as the main compounds. Limonene (1.5%) was the only highlighted monoterpene hydrocarbon. Sesquiterpene hydrocarbons (8.8%) were the second subclass of essential oil constituents with trans-caryophyllene (2.2%), germacrene D (1.4%), trans-β-farnesene (1.1%) and α-amorphene (1.1%) as their main ones. α-Cadinol (2.9%) – an oxygenated sesquiterpene – comprised notable amounts in the essential oil. An acetylated compound, namely menthyl acetate (5.6%), showed traceable amounts in the volatile oil profile. Methyl eugenol, a compound with highly appreciated amounts from most previous reports, comprised only one percent of oil. In total, the chemical and percentage composition of oil from cultivated *O. basilicum* L. from Northwest Iran was characterized as a new menthone / estragole type capable of providing these oxygenated monoterpenes for related food and pharmaceutical industries.

Key words: *Ocimum basilicum* L., Lamiaceae, essential oil constituents, GC/MS, menthone, estragole, isoneomenthol

INTRODUCTION

Common basil or sweet basil (*Ocimum basilicum* L. Fam: Lamiaceae or Labiateae) is an annual herbaceous plant with common morphological characteristics of the mint family, reaching 15–45 cm in height [1, 2]. This plant has obovate serrated opposite leaves and fragrant white or violet compound terminate flowers [2, 3]. Sweet basil is a cosmopolitan herb and aromatic plant grown nearly in all parts of the world. This plant is native of Iran and commonly grows in Azerbaijan provinces [2].

Sweet basil is a multipurpose plant with divergent applications in perfume, food, cosmetic and pharmaceutical industries [4]. Medicinally, this plant and its essential oil have long been used as immunostimulant [5], sedative, hypnotic, local anesthetic [6], anticonvulsant, antitussive, diuretic [2], carminative, galactogogue, stomachic, spasmodic [7], vermifuge [8] and platelet anti-aggregant [9]. Furthermore, different biological activities such as nematicidal, fungistatic, antifungal [5, 8], insecticidal, pesticidal [4], antiviral [7], insect repellent and antioxidant [8], have been reported for this plant. Sweet basil plant and its preparations have been used in food and oral / dental products [7], fragrances, and to treat nausea, dysentery, mental fatigue, colds, rhinitis [8], decrease of plasma lipid content, clear heartburn, to soothe the nerves, remove heat and eliminate toxins [9], as a first aid treatment for wasp stings and snake bites [8] and in traditional rituals [4].

The chemical composition of basil essential oil has been investigated since the 1930s [10], and by now more than 200 chemical components have been identified. Lawrence [11]

* Corresponding author. E-mail: hassanpouraghdam@gmail.com

has found that the main constituents of the volatile oil of basil are synthesized via two distinct biochemical pathways: phenylpropanoids like chavicol, eugenol and methyl eugenol by the shikimic acid pathway, and terpenes such as linalool by the cytosolic mevalonic acid pathway. Basil essential oil is commercially termed as essence de basilic [2].

The compositional analysis of the essential oil of sweet basil has revealed a comprehensive diversity in the oil components, and the different chemovarieties have been reported from various regions of the world. Koba et al. [4] reported four chemotypes of estragol, linalool / estragol, methyl eugenol and methyl eugenol / (E)-anethol from Togo. Seven chemotypes with major components greater than 50%, namely methyl chavicol, linalool, geraniol, linalool / methyl cinnamate, linalool / geraniol, methyl cinnamate / linalool and eugenol / linalool, were characterized from Sudan [12]. Linalool, linalool / eugenol, methyl chavicol, methyl chavicol / linalool, methyl eugenol / linalool, methyl cinnamate / linalool and bergamotene have been reported as the major chemotypes of *O. basilicum* from Mississippi, USA [13]. Italian cultivars of sweet basil were categorized in three chemotypes of linalool, linalool / methyl chavicol and linalool / eugenol [14]. Methyl eugenol and α-cubebene were reported as the main components of sweet basil oil from Turkey [8]. In a previous study from Iran, methyl chavicol and linalool were the principle components of basil oil [7]. Zamfirache et al. [5] introduced germacrene D and β-elemene as the main constituents of basil oil from Hungary. Meanwhile, linalool, (Z) cinnamic acid methyl ester, estragol, eugenol, 1,8-cineol, bergamotene, methyl cinnamate, α-cadinol and limonene have been listed as major and predominant constituents of sweet basil oil from China, Croatia, Israel, Republic of Guinea, Nigeria, Egypt, Pakistan and Malaysia [6, 9, 12, 15–21].

However, only limited studies have been conducted so far on the compositional analysis of *O. basilicum* L. from Northwest Iran. The aim of the present study was to characterize for the first time the volatile oil composition of an endemic-cultivated *O. basilicum* herb from Northwest Iran.

EXPERIMENTAL

Plant material sampling and preparation. The flowering above-ground parts of endemic purple green-leaved cultivated *O. basilicum* L. plants from Maragheh district in Northwest Iran were harvested in early summer 2008. The plant specimen was identified by a plant taxonomist, and a voucher specimen was deposited in the Herbarium of the Faculty of Agriculture, University of Maragheh, Iran. The collected plant material from about 20 individual plants as spontaneous representatives of the local native population were dried at room temperature (about 30 °C) for 4–5 days until constant weight. The air-dried plant material was mixed and pulverized to obtain a homogeneous fine-grade material.

Recovery of essential oil. A sample (50 g) of air-dried powdered plant material was extracted by the hydrodistillation technique within 3 hours in an all-glass Clevenger type apparatus. The extracted crude essential oil was dried over anhydrous sodium sulphate and stored in a hermetically sealed glass flask with a rubber lid, covered with aluminum foil to protect the contents from photo-conversion and kept under refrigeration at 4 °C until analysis. Extraction was carried out in triplicate.

Gas chromatography / mass spectrometry. The analysis of the oil was carried out using a GC (Agilent Technologies 6890N) connected to a mass-selective detector (MSD, Agilent 5973B) equipped with an non-polar Agilent HP-5-MS (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 μm film thickness). The carrier gas was helium with a linear velocity of 1 ml/min. The oven temperature was set at 50 °C for 2 min, then programmed until 110 °C at the rate of 10 °C/min with a hold time of 3 min, again heated to 200 °C at the rate 10 °C/min with a 2-min hold time, and finally increased at the rate 20 °C/min to 280 °C and kept isothermal at this temperature for 2 min. The injector and detector temperatures were 300 °C and 200 °C, respectively. Injection mode, split; split ratio, 1 : 100, volume injected, 4 μl of the oil. The MS operating parameters were as follows: ionization potential, 70 eV; interface temperature, 200 °C; and acquisition mass range, 50–800.

Identification and quantification of constituents. The relative percentage amounts of the essential oil components were evaluated from the total peak area (TIC) by the apparatus software. The identification of components in the volatile oil was based on a comparison of their mass spectra and retention time with literature data and by computer matching with NIST and WILEY library as well as by comparison of the fragmentation pattern of the mass spectral data with those reported in the literature [22].

RESULTS AND DISCUSSION

Water-distillation of the *Ocimum basilicum* L. plants provided a greenish yellow lighter than water liquid with a yield of 0.7% (v/w) of the dry weight of aerial parts. The identified essential oil components, their percentage composition, retention indices and molecular formulae as well as the main classes, subclasses and chemical groups are listed in Tables 1 and 2, respectively. Forty seven components were identified in the *O. basilicum* oil, accounting for 97.9% of total oil. Monoterpenoids (77.8%) were the chief class of components, followed by a minor share of sesquiterpenoids (12.8%) and some other components (Table 2). Oxygenated monoterpenes (75.3%) were found to be the major components of the essential oil; they are characterized by the presence of high amounts of menthone (33.1%), estragol (21.5%), isoneomenthol (7.5%), menthol (6.1%), pulegone (3.7%) and linalool (1.7%). Menthone and estragol (sum 54.6%) comprised about 55% of the total oil. Limonene (1.5%) was the only representative of monoterpene hydrocarbons with relatively high amounts (Table 1). Sesquiterpene hydrocarbons (8.8%)

were the main subclass of 15 carbon sesquiterpenoidal compounds with trans-caryophyllene (2.2%), germacrene D (1.4%), trans-β-farnesene (1.1%) and α-amorphene (1.1%) as the most abundant components. α-Cadinol (2.9%), an oxygenated sesquiterpene, had the highest amount of its subclass (Table 1). From the chemical point of view, alcohols (42%) were the predominant group of compounds, followed by ketones (37.8%), acetates (5.9%), methylated compounds (1.1%) and oxides (0.8%) (Table 2). Estragol, isoneomenthol, menthol, α-cadinol, methyl eugenol and linalool were the principle members of the alcoholic constituents. Menthone and pulegone were the most important ketone compounds (Tables 1 and 2). The highlighted representative of acetylated compounds was menthyl acetate (5.6%). Methyl eugenol was the principal constituent of methylated compounds.

Table 1. **Essential oil composition of *Ocimum basilicum* L. from Iran**

Compound	RI	Molecular formula	%
α-Pinene	0 939	$C_{10}H_{16}$	0.1
Sabinene	0 975	$C_{10}H_{16}$	0.1
β-Pinene	0 979	$C_{10}H_{16}$	0.3
β-Myrcene	0 991	$C_{10}H_{16}$	0.1
3-Octanol	0 991	$C_8H_{18}O$	0.1
α-Phellandrene	1 003	$C_{10}H_{16}$	0.1
p-Cymene	1 025	$C_{10}H_{14}$	0.1
Limonene	**1 029**	**$C_{10}H_{16}$**	**1.5**
1,8-Cineole	1 031	$C_{10}H_{18}O$	0.2
(Z)-β-Ocimene	1 037	$C_{10}H_{16}$	0.1
γ-Terpinene	1 060	$C_{10}H_{16}$	0.2
Fenchone	1 078	$C_{10}H_{16}O$	0.3
Linalool	**1 097**	**$C_{10}H_{18}O$**	**1.7**
cis-Rose oxide	1 108	$C_{10}H_{18}O$	0.2
Camphor	1 146	$C_{10}H_{16}O$	0.3
Menthone	**1 153**	**$C_{10}H_{18}O$**	**33.1**
Menthol	**1 172**	**$C_{10}H_{20}O$**	**6.1**
Iso-neomenthol	**1 187**	**$C_{10}H_{20}O$**	**7.5**
Estragol	**1 196**	**$C_{10}H_{12}O_2$**	**21.5**
Pulegone	**1 237**	**$C_{10}H_{12}O_2$**	**3.7**
Chavicol	1 250	$C_9H_{10}O$	0.1
Piperitone	1 253	$C_{10}H_{16}O$	0.3
Isopulegol acetate	1 278	$C_{12}H_{20}O_2$	0.3
Menthyl acetate	**1 295**	**$C_{12}H_{22}O_2$**	**5.6**
Carvacrol	1 299	$C_{10}H_{14}O$	0.2
α-Cubebene	1 351	$C_{15}H_{24}$	0.1
Eugenol	1 359	$C_{10}H_{12}O_2$	0.1
α-Copaene	1 377	$C_{15}H_{24}$	0.5
β-Bourbonene	1 388	$C_{15}H_{24}$	0.1
β-Cubebene	1 388	$C_{15}H_{24}$	0.3
β-Elemene	1 391	$C_{15}H_{24}$	0.5
Methyl eugenol	**1 404**	**$C_{11}H_{14}O_2$**	**1**
trans-Caryophyllene	**1 419**	**$C_{15}H_{24}$**	**2.2**
trans-α-Bergamotene	1 435	$C_{15}H_{24}$	0.7
α-Guaiene	1 440	$C_{15}H_{24}$	0.1
trans-β-Farnesene	**1 457**	**$C_{15}H_{24}$**	**1.1**
Germacrene D	**1 485**	**$C_{15}H_{24}$**	**1.4**
α-Amorphene	**1 485**	**$C_{15}H_{24}$**	**1.1**
(E)-β-Ionone	1 489	$C_{13}H_{20}O$	0.1
Bicyclogermacrene	1 500	$C_{15}H_{24}$	0.5
cis-Calamene	1 540	$C_{15}H_{24}$	0.2
Spathulenol	1 578	$C_{15}H_{24}O$	0.4
Caryophyllene oxide	1 583	$C_{15}H_{24}O$	0.4
Muurolol	1 646	$C_{15}H_{26}O$	0.1
β-Eudesmol	1 651	$C_{15}H_{26}O$	0.2
α-Cadinol	**1 654**	**$C_{15}H_{26}O$**	**2.9**
Phytol	1 943	$C_{20}H_{40}O$	0.1
Total			97.9

Compounds are reported according to their elution order on non-polar column.

The essential oil of *O. bsilicum* has been the subject of former studies [4–9, 12–21]. Taking into account the chemical profile, especially the monoterpenoidal profile of the present study and reports of other scientists from different countries, it seems that there are meaningful qualitative and quantitative differences among volatile oil components. These differences are more pronounced in regard to menthone, menthol, isoneomenthol and pulegone since these oxygenated monoterpenes are characteristic of mint plants and their high amounts in basil oil are unfamiliar. To our knowledge, the presence of such high amounts of menthone and other menthane skeleton compounds in sweet basil volatile oil have not yet been reported. Meanwhile, a high amount of limonene chemotaxonomically relates *O. basilicum* to Rutaceae plants. On the other hand, low amounts of methyl eugenol and linalool weaken the chemo-similarity of the study plant with most of the above-cited literature [4–9, 12–21]. In total, it is noteworthy that, although *O. basilicum* from different localities has been thoroughly investigated with regard to volatile oil composition, the results of our findings are supportive of the concept that continuing the chemical inventory of this plant is still a major scientific interest to encourage the comprehensive exploitation of this valuable medicinal and aromatic plant. These great chemical variations from diverse localities seem to be due to the divergent climatological and geographical conditions (light quality and quantity, soil characteristics, water and nutrient availability,

Table 2. **Main classes, subclasses and chemical groups of *Ocimum basilicum* L. essential oil constituents from Iran**

Class, subclass and chemical group of compound		%
Monoterpenoids		77.8
	Monoterpene hydrocarbons	2.6
	Oxygenated monoterpenes	75.3
Sesquiterpenoids		12.8
	Sesquiterpene hydrocarbons	8.8
	Oxygenated sesquiterpenes	4
Others		7.3
Total identified		97.9
Chemical groups		
	Alcohols	42
	Ketones	37.8
	Acetates	5.9
	Methylated compounds	1.1
	Oxides	0.8

temperature fluctuation) as well as different genetical factors such as subspecies, natural hybridization and chemovariety. Furthermore, the effects of varied growing conditions like fertilization, irrigation regime, weed control and other minor factors on plant and its biochemical potential are inevitable. These different conditions and options regularly modify the photosynthesis capability and hence interactive relationship between the primary and secondary metabolism of plants and lead to the biosynthesis of distinct end-products and chemical components from the same initial substrates of aromatic principles, i. e. phenylalanine for phenylpropanoids and geranyl pyrophosphate for terpenoids. This trend, beside different harvesting time, plant parts used for extraction and the extraction protocol, strongly affect the chemical profile and eventually the biological activity of aromatic herbs and their suitability in related industries. It is possible that *O. basilicum* L. plants studied in the present experiment might be a unique chemotype of this plant owing to its distinct volatile oil composition. However, this plea requires comparative studies based on detailed phytochemical assays.

CONCLUSIONS

In brief, the chemical composition of the essential oil of cultivated *O. basilicum* L. plant from Northwest Iran was characterized by the presence of appreciable amounts of menthone and estragole. The results showed substantial chemical profile differences between the present study and previous reports. However, it can be noted that *O. basilicum* plants studied in the current study could be a good source of these oxygenated monoterpenes in supplying the increasing demands of food, cosmetic and pharmaceutical industries.

Received 2 November 2009
Accepted 23 November 2009

References

1. A. Zarghari, *Medicinal Plants* (in Persian), Tehran University Publications, Iran (1997).
2. V. Mozaffarian, *A Dictionary of Iranian Plant Names* (in Persian), Farhang Moaaser Publishing Company, Iran (2004).
3. A. Ghahreman, *Plant Systematics: Cormophytes of Iran* (in Persian), Iran University Press, Iran (1993).
4. A. Koba, P. W. Poutouli, C. Raynaud, J. P. Chaumont, K. Sanda, *Bangladesh J. Pharmacol*., **4**, 1 (2009).
5. M. M. Zamfirache, I. Burzo, Z. Olteanu, S. Dunca, S. Surdu, E. Truta, M. Stefan, C. M. Rosu, *An. St. Univ. Al. L. Cuza. Iasi*, **4**, 35 (2008).
6. M. Ismail, *Pharm. Biol*., **44(8)**, 619 (2006).
7. S. E. Sajjadi, *Daru*, **14(3)**, 128 (2006).
8. M. Ozcan, J. C. Chalchat, *Czech J. Food Sci*., **20(6)**, 223 (2002).
9. J. W. Zhang, S. K. Li, W. J. Wu, *Molecules*, **14**, 273 (2009).
10. X. Chang, P. G. Alderson, C. J. Wright, *Environ. Exp. Bot*., **63**, 216 (2008).
11. B. M. Lawrance, in: B. M. Lawrance, B. D. Mokheyee, B. J. Willis (eds.), *Developments in Food Sciences, Flavour and Fragrances; A World Perspective,* Elsevier, the Netherlands (1988).
12. A. H. N. Abduelrahman, E. A. Alhussein, N. A. I. Osman, A. H. Nour, *Int. J. Chem. Technol*., **1(1)**, 1 (2009).
13. V. D. Zheljazkov, A. N. Callahan, C. L. Cantrell, *J. Agric. Food Chem*., **56(1)**, 241 (2007).
14. M. Marotti, R. Piccaglia, E. Giovanelli, *J. Agric. Food Chem*., **44(12)**, 3926 (1996).
15. E. Werker, E. Putievsky, U. Ravid, N. Dudai, I. Katzir, *Ann. Bot*., **71**, 43 (1993).
16. O. Politeo, M. Jukic, M. Milos, *Croat. Chem. Acta*, **79(4)**, 545 (2006).
17. S. M. Keita, C. Vincent, J. P. Schmit, A. Belanger, *Flavour Frag. J*., **15(5)**, 339 (2000).
18. A. A. Kasali, A. O. Eshilokun, S. Adeola, P. Winterhalter, H. Knapp, B. Bonnlander, *Flavour Frag. J*., **20(1)**, 45 (2004).
19. A. J. Hussein, F. Anwar, S. T. H. Sherazi, R. Prybylski, *Food Chem*., **108(3)**, 986 (2008).
20. J. C. Chalchat, M. M. Ozcan, *Food Chem.*, **110(2)**, 501 (2008).
21. S. R. Vani, S. F. Cheng, C. H. Chuah, *Am. J. Appl. Sci*., **6(3)**, 523 (2009).
22. R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, Allured Publishing Corp., Carol Stream, IL, USA (2004).

Mohammad Bagher Hassanpouraghdam, Abbas Hassani, Mohammad Safi Shalamzari

MENTONO IR ESTRAGOLIO TURINTIS ETERINIS ALIEJUS IŠ ŠIAURĖS VAKARŲ IRANE KULTIVUOJAMO *OCIMUM BASILICUM* L.

S a n t r a u k a

Eterinis aliejus, hidrodistiliavimo būdu išskirtas iš Šiaurės vakarų Irane kultivuojamų *Ocimum basilicum* L. augalų, buvo tiriamas dujų chromatografijos-masių spektrometrijos metodu. Buvo identifikuoti 47 komponentai, kartu sudarantys 97,9 % eterinio aliejaus. Tarp komponentų rasta 77,8 % monoterpenoidų ir 12,8 % seskviterpenoidų. Apskritai tirtasis eterinis aliejus yra apibūdinamas kaip naujo mentono–estragolio tipo aliejus, kuris gali būti panaudotas maisto ir farmacijos pramonėje.