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# Chemical composition and yield of essential oil from two Iranian species of basil (*Ocimum ciliatum* and *Ocimum basilicum*)

Abdollah Ghasemi Pirbalouti[1,3] ✉, Fatemeh Malekpoor[1,2] and Azam Salimi[2]

[1]Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, 88146, Iran
[2]Department of Plant Biology, Faculty of Biological Science, Kharazmi University, 15719-14911, Tehran, Iran
[3]Medicinal Plants Program, College of Natural Sciences, Massachusetts University, Amherst, 01003, MA, USA

## ABSTRACT

Basil belonging to the Lamiaceae family, is one of the most important aromatic and medicinal plants, and is widely cultivated in many countries. Areal parts, especially leaves of sweet basil are widely used to enhance the flavour of food stuffs such as salads, pasta, tomato products, vegetables, pizza, meat, soups, marine foods, confectioneries and other products. In this report, the yield and chemical components of essential oils of two species of basil (*Ocimum ciliatum* and *Ocimum basilicum*) grown in southwestern of Iran (Chaharmahal and Bakhtiary Province) were investigated. The water-distilled oils were analyzed by means of a GC-MS apparatus. The oil yields obtained from the aerial parts of *Ocimum basilicum* were found to be 0.285 mL/100 g and 0.71 mL/100 g when using the dry plant materials, respectively. The major constituents of the essential oil from the aerial parts of *O. basilicum* were methyl chavicol or estragol (49.7%), linalool (10.7%), α-cadinol (5.9%), (*Z*)-β-farnesene (3.8%) and 1,8-cineole (3.5%). The main constituents of the essential oil from the aerial parts of *O. ciliatum* were methyl chavicol (38.1%), (*E*)-citral (14.6%), (*Z*)-citral (11.5%), (*E*)-caryophyllene (6.4%) and *cis*-α-bisabolene (4.0%). A simple comparison of our results with the previous reports suggests that differences in the essential oil compositions and oil yields of the studied plants could be mainly attributed to genetic diversity in two Iranian species of basil.



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## 1. Introduction

*Ocimum* L. (basil) is considered as one of the largest genera of the Lamiaceae family and comprises annual, perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa, and South America (Moghaddam et al., 2014). Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, kidney malfunctions, heart problems, and abdominal pains (Simon et al., 1999; Lee et al., 2005). As a potential source of aroma compounds, the essential oils and extracts from different species of basil have shown remarkable effects including amnesic (Singh et al., 2016) and relaxant (Shakeri and Boskabady, 2015) impacts. They also possess a broad range of biological activities, including insect repellent and nematocidal (Anees, 2008; Ogendo et al., 2008), antibacterial (Suppakul et al., 2003; Oxenham et al., 2005; Carovic-Stanko et al., 2010; Sharafati-Chaleshtori et al., 2015; Silva et al., 2016), antioxidant (Juliani and Simon, 2002; Lee et al., 2005; Trevisan et al., 2006; Sharafati-Chaleshtori et al., 2015) antifungal (Soares et al., 2015), and hepatoprotective (Soliman et al., 2016) properties.

Most culinary and ornamental basils are cultivars of sweet basil (*O. basilicum*), which is widely cultivated in many countries. Among the Iranian basil species, *O. ciliatumas* as the main species of Iranian herbs (Makari and Kintzios, 2008; Moghaddam et al., 2015) is grown in home gardens and the related leaves and herbaceous parts of this medicinal plant are used as

✉ Corresponding author: Abdollah Ghasemi Pirbalouti
Tel: +98-3113913348, +1-413 545 2570, Fax: +98-3113912254, +1-413 545 3958
E-mail address: ghasemi955@yahoo.com, ghasemi@iaushk.ac.ir

vegetables and for culinary purposes (Moghaddam et al., 2011).

The essential oil from the aerial parts of basil consists of a wide and varying array of chemical constituents, depending on variations in chemotypes, leaf and flower colors, aroma, and origin of the plants (Javanmardi et al., 2003; Sajjadi, 2006; Chalchat and Ozcan, 2008; Carovic-Stanko et al., 2010; Ghasemi Pirbalouti et al., 2013a; Smitha and Tripathy, 2016; Mohammadhosseini, 2016; Mohammadhosseini, et al., 2017). Methyl chavicol, methyl cinnamate, methyl eugenol, citral, and linalool are generally considered among the main chemotypes of basil. In addition, basil has been reported to contain high contents of monoterpenoids (carvone, cineole, fenchone, geraniol, linalool, myrcene and thujone), sesquiterpenoids (caryophyllene and farnesol), a triterpenoid (ursolic acid), and a flavonoid (apigenin) (Ghasemi Pirbalouti et al., 2013a). Numerous investigations on the chemical compositions of the essential oils of basil (Sajjadi, 2006; Carovic-Stanko et al., 2010), however, have demonstrated a considerable variability within its diverse species. Various researchers reported that yields of the essential oils and the respective profiles in medicinal and aromatic plants are remarkably related to their genetic (Shafie et al., 2009), climate, edaphic, elevation and topography (Pourohit and Vyas, 2004; Loziene and Venskutonis, 2005; Rahimmalek et al., 2009; Ghasemi Pirbalouti et al., 2013a, b, c; Ghasemi Pirbalouti, 2014; Padalia et al., 2017), as well as their possible interactions (Basu et al., 2009). Recent findings on medicinal plants indicated that some of their characteristics can be greatly affected by genetic and ecological factors, including precipitation, temperature, plant composition, and nitrogen content in the soil (Ghasemi Pirbalouti et al., 2013b). The main goal of this study was to determine the variation of chemical composition and yields of the essential oils from the aerial parts of two Iranian species of basil, namely *O. ciliatum* and *O. basilicum*.

## 2. Experimental

### 2.1. Plant materials

The experiments were conducted at the Research Field, Islamic Azad University of Shahrekord (latitude: 32º 20' N; longitude: 50º 51' E; altitude: 2070 msl), southwestern Iran, in May-August 2014. Seeds of two species of basil were sown in plastic pots with a diameter of 20 cm and a depth of 25 cm. The climate of the sampling area study is cold, semiarid and semi humid with temperate summer by Emberger's climatology method and very cold winter by Karimi's climatology method (IRIMO, 2012). The pots were filled with clay loam with a pH of 7.23 containing 0.8% organic matter consisted of 0.01% total N, 11.20 mg/kg available phosphorus, 694 mg/kg available potassium, and a saline value measured at E.C.= 1.35 dS/m. The aerial parts of the two Iranian species of basil including *O. ciliatum* and *O. basilicum* were collected before the flowering stage. The plants were cut from 1 cm area just above the lignified parts of the stem, and then dried under the room temperature. The gathered plant samples were first confirmed by Mrs. Salimi and voucher specimen (IAUSHK-81G and IAUSHK-81P) have been deposited in the Herbarium of Research Center of Medicinal Plants, Shahrekord Branch, I.A.U. Iran.

### 2.2. Essential oil extraction

Dried parts of *Ocimum* species (100 g) were powdered and subjected to hydrodistillation for three hours using a Clevenger-type apparatus. The obtained essential oils were dried with anhydrous sodium sulphate and kept in amber vials at 4 ºC prior to use.

### 2.3. Identification of the oil components

Chemical compositions of the essential oils of the two *Ocimum* species were determined by gas chromatography (GC) and gas chromatography combined with mass spectrophotometry (GC/MS). The GC analysis was performed on an Agilent technologies 7890 GC (Santa Clara, CA) equipped with a single injector and a flame ionization detector (FID). A polar HP Innowax column and an apolar HP-5 capillary column (30 m×0.25 mm, 0.25 μm film thickness) coated with 5% phenyl, 95% methyl polysiloxane were used. The flow-rate of the carrier gas ($N_2$) was 0.8 mL/min. Initial column temperature was 60 ºC and programmed to increase with a 4 ºC/min ramp to a final temperature of 280 ºC. The injector temperature was finally set at 300 ºC. Split injection was conducted with a ratio split of 1:40. Then, 0.1 μL portions of the separated essential oil samples were directly injected onto the injection port of the apparatus. GC-MS analyses of aromatic oil samples were performed on an Agilent technologies 7890 gas chromatograph coupled to Agilent 5975 C mass selective detector (MSD) and quadrupole EI mass analyzer. An HP-5MS 5% column coated with methyl silicone (30 m×0.25 mm, 0.25 μm film thickness) was used as the stationary phase. Helium was used as the carrier gas at a 0.8 mL/min flow-rate. The temperature was programmed from 60 ºC to 280 ºC using a 4 ºC/min ramp rate. The injector and the GC-MS interface temperatures were maintained at 290 ºC and 300 ºC, respectively. All the mass spectra were recorded at 70 eV over a mass range of 50-550 (m/z). The ion source and the detector temperatures were maintained at 250 ºC and 150 ºC, respectively. The oil constituents were identified based on: i) their retention indices which were determined with reference to a homologous series of $C_5$-$C_{24}$ of *n*-alkanes), ii) through comparison of their mass spectral patterns with those reported in the literature (Adams, 2007) and stored in NIST 08 (National Institute of Standards and Technology) as well as Willey (ChemStation data system) libraries. The peak area percentages were computed from an HP-5 column without the use of FID response factors.

2.4. Statistical analyses

The data was statistically analyzed using a completely randomized design (CRD) using SPSS (19.0) software. Means of the main constituents of the essential oils were compared by Duncan's multiple range test at $p<0.05$ level.

## 3. Results and Discussion

3.1. Essential oil yield

The essential oils extracted from the aerial parts of the two Iranian species of basil produced clear, and yellowish liquids. A significant difference ($p<0.05$) in oil yields was obtained from the aerial parts of these plants. The yields of the obtained volatile oils from the aerial parts of *O. ciliatum* and *O. basilicum* were found to be 0.71 mL/100 g and 0.285 mL/100 g respectively with respect to the dry matter. An earlier study by Moghaddam et al. (2014) reported that the essential oil yields of *O. ciliatum* in different phenological stages were over the range 0.80-1.80%. In another study, Moghaddam et al. (2015) reported the essential oil content of *O. ciliatum* being 1.3% (w/w). The study of Sajjadi (2006) indicated the oil yields from the aerial parts of *O. basilicum* cv. purple and *O. basilicum* cv. Green collected at full flowering stage from the same region were 0.2% and 0.5% (v/w), respectively. In addition, Tahsili et al. (2010) measured the oil yield of the aerial parts of *O. basilicum* collected from Alborz Province, Iran, as 0.75% (v/w) before the flowering period. The essential oil yield from the green landrace was higher than the purple landrace, as well. Earlier reports by Ghasemi Pirbalouti (2014) showed significant differences of the essential oil yields between two Iranian landraces of basil. Variations in the quality and quantity of essential oils from medicinal and aromatic plants are significantly influenced by intrinsic parameters, including species, chemotype/genotype, genetic structures, anatomical different plant parts, organogenesis and growth stages (Nemeth, 2005). In addition, external factors such as ecological and environmental conditions, including season, climatic and soil conditions (Nejad Ebrahimi et al., 2008), extraction and drying methods (Ghasemi Pirbalouti et al., 2013a; Msaada et al., 2007) had significant effects on the quality and quantity of the essential oils from aromatic plants.

3.2. Chemical composition of the basil oils

The results of characterization of the essential oils of *O. ciliatum* and *O. basilicum* using the GC/FID and GC/MS analyses are shown in Table 1. As can be seen from this table, 30 and 36 components were determined in the essential oils of *O. ciliatum* and *O. basilicum*, respectively totally accounting for 95.6% and 94.4% of the oil profiles (Table 1). The major constituents of the essential oil from the aerial parts of *O. basilicum* (Fig. 1) were methyl chavicol or estragol (49.7%), linalool (10.7%), α-cadinol (5.9%), (*Z*)-β-farnesene (3.8%) and 1,8-cineole (3.5%). The main natural compounds in the essential oil from the aerial parts of *O. ciliatum* (Fig. 2) were methyl chavicol (38.1%), (*E*)-citral (14.6%), (*Z*)-citral (11.5%), (*E*)-caryophyllene (6.4%) and *cis*-α-bisabolene (4.0%). Chemical compositions of basil essential oils have been the subjects of some previous reports (Hiltunen and Holm, 1999; Lee et al., 2005; Telci et al., 2006; Vieira and Simon, 2006; Pirmoradi et al., 2013). Accordingly, there is usually an extensive diversity in the major

(a) (b)

**Fig. 1.** Photographs of *Ocimum* species (a) *Ocimum basilicum* and (b) *Ocimum ciliatum*.

**Table 1**
Chemical composition of the essential oils of *Ocimum ciliatum* and *Ocimum basilicum*.

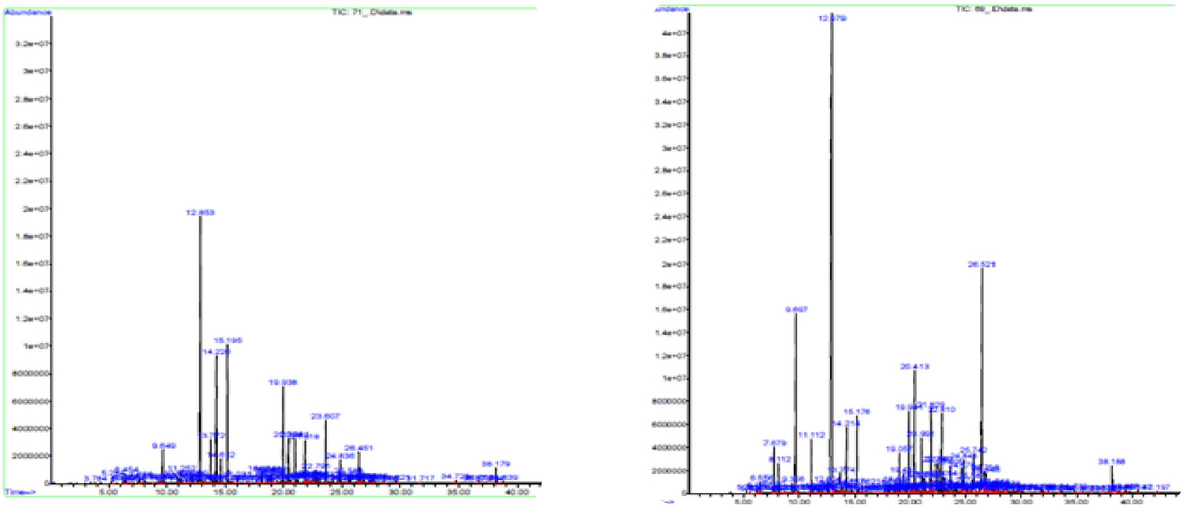
| No | Component | RI | Percentage | |
|---|---|---|---|---|
| | | | ***O. ciliatum*** | ***O. basilicum*** |
| 1 | α-Pinene | 936 | 0.5 | 0.2 |
| 2 | Camphene | 950 | - | 0.1 |
| 3 | Sabinene | 974 | 0.04 | 0.2 |
| 4 | β-Pinene | 979 | 0.2 | 0.3 |
| 5 | Myrcene | 992 | 0.1 | 0.8 |
| 6 | Limonene | 1028 | 0.2 | 0.3 |
| 7 | 1,8-Cineole | 1031 | 0.1 | **3.5** |
| 8 | (*E*)-β-Ocimene | 1048 | 0.04 | 0.2 |
| 9 | (Z)-β-Ocimene | 1054 | - | 2.4 |
| 10 | Linalool | 1098 | 0.3 | **10.7** |
| 11 | Camphor | 1110 | - | 1.4 |
| 12 | Borneol | 1154 | - | 0.2 |
| 13 | Terpinen-4-ol | 1174 | - | 0.1 |
| 14 | α-Terpineol | 1188 | 0.1 | 0.4 |
| 15 | Methyl chavicol | 1210 | **38.1** | **49.7** |
| 16 | Nerol | 1239 | 3.4 | - |
| 17 | (*Z*)-Citral | 1254 | **11.5** | 0.2 |
| 18 | Geraniol | 1272 | 1.7 | - |
| 19 | (*E*)-Citral | 1285 | **14.6** | 0.2 |
| 20 | Bornyl acetate | 1314 | - | 0.2 |
| 21 | Neryl acetate | 1355 | 1.4 | - |
| 22 | α-Copaene | 1369 | 0.7 | - |
| 23 | Geranyl acetate | 1379 | 0.5 | - |
| 24 | β-Bourbonene | 1381 | 0.7 | 0.2 |
| 25 | β-Cubebene | 1384 | 0.8 | - |
| 26 | β-Elemene | 1386 | - | 1.2 |
| 27 | Eugenol | 1404 | 0.2 | 0.2 |
| 28 | (*E*)-Caryophyllene | 1414 | **6.4** | 1.8 |
| 29 | α-Bergamotene | 1435 | 2.0 | - |
| 30 | α-Humulene | 1447 | 2.4 | 1.5 |
| 31 | Germacrene D | 1456 | 1.3 | 2.0 |
| 32 | (*Z*)-β-Farnesene | 1465 | 0.2 | **3.8** |
| 33 | *epi*-Bicyclosesquiphellandren | 1472 | - | 0.4 |
| 34 | Bicyclogermacrene | 1489 | - | 1.0 |
| 35 | β-Eudesmene | 1495 | - | 1.1 |
| 36 | δ-Cadinene | 1516 | 0.2 | 2.2 |
| 37 | Calamenene | 1520 | - | 0.3 |
| 38 | *cis*-α-Bisabolene | 1542 | **4.0** | 0.2 |
| 39 | Nerolidol | 1564 | - | 0.1 |
| 40 | Spathulenol | 1570 | - | 0.6 |
| 41 | Caryophyllene oxide | 1574 | 3.4 | 0.5 |
| 42 | α-Cadinol | 1585 | 0.3 | 5.9 |
| 43 | T-Muurolol | 1589 | 0.2 | 0.3 |
| | | | **95.6** | **94.4** |

compounds within the *Ocimum* spp. A simple comparison of our results with those available in the literature indicated that the characterized profiles had considerable differences with the other species of this genus. To date, several chemotypes have been established in various phytochemical investigations on some *Ocimum* species. The recurring polymorphism determines a large number of subspecies, different varieties and forms producing essential oils with varying chemical composition. In most cases, oxygenated monoterpenes (linalool, neral, geranial, geraniol, camphor, 1,8-cineole and thymol) and phenylpropanoid (methyl chavicol, methyl cinnamate, eugenol and methyl eugenol) have been reported as the major components of the essential oils in this genus in varying degrees of abundance (Hiltunen and Holm, 1999; Lee et al., 2005). Phenylpropane derivatives were previously reported in the essential oils of *O. basilicum*, including methyl chavicol (Ghasemi Pirbalouti et al., 2013a), methyl eugenol and eugenol (Lewinsohn et al., 2000) along with methyl cinnamate (Simon et al., 1999). In a related study, the major compounds were phenylpropane derivatives or terpenoids, including methyl eugenol and 1,8-cineole (Runyoro et al., 2010). Moghaddam et al. (2015) reported 23 components in the essential oils of *O. ciliatum*, representing about 98% of all components in the oil. The major constituting compounds in this study were methyl chavicol (87.6%), methyl eugenol (2.6%), and 1,8-cineole (1.7%) making the essential oil of *O. ciliatum* as a potential industrial source of methyl chavicol. Similarly, Sajjadi (2006) identified 20 constituents in the volatile oil of *O. basilicum* cv. Purple collected at full flowering stage. In accordance with this study, the main constituents were methyl chavicol (52.4%), linalool (20.1%), *epi*-α-cadinol (5.9%) and *trans*-α-bergamotene (5.2%). Furthermore, 12 components were totally characterized in the volatile oil of *O. basilicum* cv. green collected at full flowering step with methyl chavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%) as the major components. A comparison of our results with the previous report of Sajjadi (2006) suggests that a few dissimilarities in the volatile composition of the plant material could be attributed to: i) the growth and cultivation conditions of the plant, ii) the methods of extraction and iii) the harvesting time.

Moreover, results of this study indicated significant differences ($p$<0.01) among the main constituents in the essential oils from the aerial parts of two Iranian species of basil. The observed variability of the main constituent contents in these basil oils are mainly attributed to different genotypes (species and ecotype) and/or chemotypes (Ghasemi Pirbalouti et al., 2013b, c).

## 4. Concluding remarks

The results of this report provide further data on variation of the essential oils profiles from two Iranian species of basil. The present study indicates that the essential oil components of two basil plants (*O. ciliatum* and *O. basilicum*) vary greatly depending on their genotypes and chemotypes. Phenylpropanoids and oxygenated monoterpenes were the main constituents of the corresponding essential oils. Characterization of the oil profiles also indicated that methyl chavicol or estragol, linalool, α-cadinol, (*Z*)-β-farnesene and 1,8-cineole for the *O. basilicum* and methyl chavicol, (*E*)-citral, (*Z*)-citral, (*E*)-caryophyllene



(a) (b)

**Fig. 2.** GC-MS chromatograms of the essential oil of *Ocimum* species: (a) *Ocimum basilicum* (b) *Ocimum ciliatum*.

and *cis*-α-bisabolene for the *O. ciliatum* were the main constituents of the essential oils. The observed differences in the essential oil yields and their components among the studied landraces of basil could be attributed to their genetic diversity.

### Conflict of interest

The authors declare that there is no conflict of interest.

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