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## Inflorescence and leaves essential oil composition of hydroponically grown *Ocimum basilicum* L.

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**Abstract:** In order to characterize the essential oils of leaves and inflorescences, water distilled volatile oils of hydroponically grown *Ocimum basilicum* L. were analyzed by GC/EI-MS. Fifty components were identified in the inflorescence and leaf essential oils of the basil plants, accounting for 98.8 and 99.9 % of the total quantified components respectively. Phenylpropanoids (37.7 % for the inflorescence vs. 58.3 % for the leaves) were the predominant class of oil constituents, followed by sesquiterpenes (33.3 vs. 19.4 %) and monoterpenes (27.7 vs. 22.1 %). Of the monoterpenoid compounds, oxygenated monoterpenes (25.2 vs. 18.9 %) were the main subclass. Sesquiterpene hydrocarbons (25 vs. 15.9 %) were the main subclass of sesquiterpenoid compounds. Methyl chavicol, a phenylpropane derivative, (37.2 vs. 56.7 %) was the principle component of both organ oils, with up to 38 and 57 % of the total identified components of the inflorescence and leaf essential oils, respectively. Linalool (21.1 vs. 13.1 %) was the second common major component followed by  $\alpha$ -cadinol (6.1 vs. 3 %), germacrene D (6.1 vs. 2.7 %) and 1,8-cineole (2.4 vs. 3.5 %). There were significant quantitative but very small qualitative differences between the two oils. In total, considering the previous reports, it seems that essential oil composition of hydroponically grown *O. basilicum* L. had volatile constituents comparable with field grown counterparts, probably with potential applicability in the pharmaceutical and food industries.

**Keywords:** *Ocimum basilicum* L.; Lamiaceae; essential oil; hydroponic; methyl chavicol; linalool.

### INTRODUCTION

Hydroponics, the method of growing plants without soil, has long been the subject of much public interest in most parts of the world.<sup>1</sup> This method of plant

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production is an efficient alternative for countries short on water supply and limited in agricultural soil. Furthermore, in the last three decades, hydroponic culture has been employed as an economic and environmentally viable means for the mass production of vegetables and potting and/or cut flowers in most parts of the world.<sup>1</sup> Hydroponic production of medicinal and aromatic plants is a new trend in agricultural systems, particularly in organic and intensive agriculture.<sup>2-4</sup> High yields, cleaner and off-season production, balanced nutrient availability, adequate aeration and high water use efficiency have been defined as the main advantages of hydroponics production systems.<sup>2-6</sup>

Common basil (*Ocimum basilicum* L.), a herbaceous annual fragrant herb, belongs to the Lamiaceae family.<sup>7,8</sup> Basil is a cosmopolitan herb and aromatic plant with abundant applications in pharmaceutical, food and fragrance Industries.<sup>8,9</sup> In Iran, basil is a herb with great use in food products and gastronomy.<sup>9</sup> Furthermore, basil production occupies large areas of land, especially near crowded cities. Hydroponic production is a competent alternative for contending with the high demands for this crop.

Pharmaceutically, this plant and its preparations have been used for a long time as immunostimulant, sedative, hypnotic, local anesthetic, anticonvulsant, diuretic, carminative, galactagogue, stomachic, spasmodic and vermifuge purposes.<sup>7,10-15</sup> Additionally, several biological activities have been reported for its secondary metabolites, such as nematocidal, fungistatic, antifungal, insecticidal, pesticidal, antiviral, insect repellent and antioxidant.<sup>10,11,13,14</sup> Owing to aforementioned biological and healing activities, sweet basil plant and its preparations have been used to treat nausea, dysentery, mental fatigue, cold, rhinitis, increased plasma lipid content, soothe the nerves and as a first aid treatment for wasp stings and snake bites.<sup>10,13-15</sup>

The chemical analysis of basil essential oil has been investigated since the 1930s.<sup>16</sup> Lawrence<sup>17</sup> reported that the main components of basil volatile oil are synthesized *via* two distinct biochemical pathways, *i.e.*, the shikimic acid pathway leading to phenylpropane derivatives and the mevalonic acid pathway leading to terpenoid compounds. A substantial number of studies conducted on the composition of the essential oil of basil revealed a huge diversity in the constituents of its oil with different chemotypes from many regions of the world. Estragol, linalool, methyl eugenol, geraniol, methyl cinnamate, bergamotene,  $\alpha$ -cubebene, germacrene D,  $\beta$ -elemene, 1,8-cineole, methyl cinnamate,  $\alpha$ -cadinol and limonene are considered as the main constituents and chemotypes of basil from different parts of the world.<sup>10-15,18-27</sup> As mentioned above, there are several reports on the composition of the volatile oil of wild and field grown basil. In a recent study, menthone and estragol were reported to be major components of field grown basil plants from Iran.<sup>9</sup> However, to the best of our knowledge, there is scarce information on the chemical composition of the volatile oil of hydroponi-

cally grown *O. basilicum*. The objective of the present study was to comparatively characterize the composition of leaves and inflorescence essential oil of hydroponically grown *O. basilicum* L. plant from Iran.

#### EXPERIMENTAL

This experiment was conducted at the Research Greenhouse of Horticultural Sciences Department, Tabriz University, Iran, during spring–summer of 2009.

##### *Plant material*

Seeds of a native *O. basilicum* L. plants were directly sowed in 5 L pots. During the germination period and first two weeks of plantlet growth, they were irrigated with tap water. A quarter-strength modified Hoagland nutrient solution was used for regular irrigation of the plants for the following two weeks. Then after, the established plants were daily irrigated with half-strength Hoagland solution until harvest time. The pH and EC of the nutrient solution were adjusted to 6–6.5 and 2 dS m<sup>-1</sup> using H<sub>2</sub>SO<sub>4</sub> or KOH and water, respectively.<sup>2-4</sup>

This experiment was realized in a one-layer polyethylene covered greenhouse at ambient temperature, humidity and light intensity, *i.e.*, 15–30 °C, 40–50 % and 500 μmol m<sup>-2</sup> s<sup>-1</sup>, respectively. The pots were regularly watered with tap water in 10-day intervals for prevention of salinity accumulation in the growing media.<sup>2</sup> The aerial parts of 30 plants (3 plants per each pot, equally distanced for optimum light interception) were harvested at the flowering stage, dissected to leaves and inflorescences and dried at room temperature for 4–5 days.

##### *Volatile oil extraction*

Pooled samples (50 g) of air-dried powdered plant materials (inflorescence and leaves) were extracted by the hydrodistillation technique for 3 h in an all-glass Clevenger type apparatus. The extracted crude essential oils were dried over anhydrous sodium sulfate and stored in hermetically sealed glass flasks with a rubber lid, covered with aluminum foil to protect the contents from the light and air conversion and kept in a refrigerator at 4 °C until analysis.

##### *Instrumentation*

A GC/MS instrument (Agilent 6890N GC and Agilent 5973 mass selective detector operating in the EI mode, USA) was used for the volatile oil analysis. Ultra pure helium (99.99 %, Air Products, UK) passed through a molecular sieve trap and an oxygen trap (Chromatography Research Supplies, USA) was used as the carrier gas at a constant velocity of 1 ml min<sup>-1</sup>. The injection port was held at 300 °C and used in the split mode; split ratio 1:100, volume injected: 5 μl of the pure volatile oil. The detector temperature was 200 °C. Separation was realized on an apolar HP5MS (5 %-phenyl methyl poly siloxane; 30 m×0.25 mm i.d. and 0.25 μm film thickness) capillary column (Hewlett-Packard, USA). The oven temperature was programmed as follows: 50 °C (held 2 min), raised to 110 °C at a rate of 10 °C min<sup>-1</sup>, then heated to 200 °C at a rate of 10 °C/min and finally increased to 280 °C at 20 °C min<sup>-1</sup>, isothermal at this temperature for 2 min. The mass operating parameters were as follows: ionization potential: 70 eV, interface temperature: 200 °C and acquisition mass range: 50–800.

##### *Identification and quantification of volatile oil components*

The relative percentage amounts of the volatile oil constituents were evaluated from the total peak area (TIC) using instrument software. The components of the essential oil were identified by comparing their mass spectral fragmentation patterns with those of similar

compounds from the NIST and WILEY library databases, as well as by comparing their Kovats gas chromatographic retention indices with those of the literature.<sup>10-15,18-28</sup>

## RESULTS AND DISCUSSION

The hydrodistillation of the inflorescence and leaves of *O. basilicum* L. gave pale yellow liquids with a yield of 0.6 and 0.5 (v/w) based on the dry weight. The chemical composition of the studied oils, their retention indices, molecular formulae and percentage composition are presented in Table I. The main classes and subclasses of the identified components are reported in Table II as well. Fifty components were identified in the essential oil of inflorescence and leaves of hydroponically grown *O. basilicum*, accounting for 98.8 and 99.9 % of total identified components, respectively (Tables I and II). Phenylpropanoids were the main class of constituents with methyl chavicol (37.2 % in the inflorescence *vs.* 56.7 % in leaves) as their main representatives (Tables I and II). Sesquiterpenoids (33.3 *vs.* 19.4 %) were characterized as the second major class of volatile components and then monoterpenoids (27.7 *vs.* 22.1 %). Sesquiterpene hydrocarbons (25 *vs.* 15.9 %) were assigned as the main subclass of the volatile oil components (Table II). Another major subclass of components was oxygenated monoterpenes (25.2 *vs.* 18.9 %). Monoterpene hydrocarbons and oxygenated sesquiterpenes had a minor share in the essential oil profile (Table II). Considering the main classes and subclasses, there were quantitative differences between the leaves and inflorescence essential oil. In light of the major class of volatile oil components, *i.e.*, phenylpropanoids, the leaf essential oil was superior to the inflorescence essential oil (Table II). Contrarily, the inflorescence essential oil was richer in sesquiterpenoids subclasses and oxygenated monoterpenes (Table II). Methyl chavicol, a phenylpropanoid pathway product, was the principal common constituent of both organs oils. Linalool, a highly appreciated oxygenated monoterpene, (21.1 *vs.* 13.1 %) was ranked as the second most abundant common component (Table I).  $\alpha$ -Cadinol (6.1 *vs.* 3 %), germacrene D (6.1 *vs.* 2.7 %), 1,8-cineole (2.4 *vs.* 3.5 %),  $\gamma$ -cadinene (2.5 *vs.* 2 %),  $\alpha$ -(*Z*)-bergamotene (1.8 *vs.* 2.6 %), bicyclogermacrene (1.8 *vs.* 1.4 %), (*E*)- $\beta$ -farnesene (1.7 *vs.* 1.6 %), (*E*)-caryophyllene (1.4 *vs.* 1.0 %) and camphor (1.1 *vs.* 1.1 %) were the other common components of the oils (Table I).  $\alpha$ -Guaiene (5.4 *vs.* 0.4 %) and  $\beta$ -elemene (2.1 *vs.* 0.7 %) were defined as two other common components with superiority of the inflorescence oil. (*E*)- $\beta$ -Ocimene (0.9 *vs.* 1.2 %) and eugenol (0.3 *vs.* 1 %) were common components with higher amounts in the leaf oil than in the inflorescence oil (Table I). Spathulenol (1.3 %) and  $\alpha$ -bulnesene (1.2 %) were constituents exclusive to the inflorescences and leaves, respectively (Table I). From chemical standpoint, alcohols were the highlighted components of both oils with notable amounts of linalool and  $\alpha$ -cadinol. Taking into account the major volatile oil constituents, there was a significant quantitative but no strong qualitative difference between the two oils.

TABLE I. Chemical composition of the essential oil of hydroponically grown *O. basilicum* L. (compounds are reported according to their elution order on a non-polar column)

No.	Compound	RI	Molecular formula	Peak area, %	
				Inflorescence	Leaves
1	$\alpha$ -Pinene	0939	C <sub>10</sub> H <sub>16</sub>	0.1	0.2
2	Camphene	0954	C <sub>10</sub> H <sub>16</sub>	0.1	0.1
3	Sabinene	0975	C <sub>10</sub> H <sub>16</sub>	0.1	0.1
4	$\beta$ -Pinene	0979	C <sub>10</sub> H <sub>16</sub>	0.4	0.6
5	Myrcene	0991	C <sub>10</sub> H <sub>16</sub>	0.3	0.7
6	$\alpha$ -Terpinene	1017	C <sub>10</sub> H <sub>16</sub>	–	0.1
7	Limonene	1029	C <sub>10</sub> H <sub>16</sub>	0.2	–
8	1,8-Cineole	1031	C <sub>10</sub> H <sub>18</sub> O	2.4	3.5
9	(Z)- $\beta$ -Ocimene	1037	C <sub>10</sub> H <sub>16</sub>	–	0.1
10	(E)- $\beta$ -Ocimene	1050	C <sub>10</sub> H <sub>16</sub>	0.9	1.2
11	$\gamma$ -Terpinene	1060	C <sub>10</sub> H <sub>16</sub>	0.1	0.1
12	(Z)-Sabinene hydrate	1070	C <sub>10</sub> H <sub>18</sub> O	–	0.1
13	Fenchone	1087	C <sub>10</sub> H <sub>16</sub> O	–	0.8
14	Terpinolene	1089	C <sub>10</sub> H <sub>16</sub>	0.3	–
15	Linalool	1097	C <sub>10</sub> H <sub>18</sub> O	21.1	13.1
16	Camphor	1146	C <sub>10</sub> H <sub>16</sub> O	1.1	1.1
17	Borneol	1169	C <sub>10</sub> H <sub>18</sub> O	0.4	0.2
18	Terpinene-4-ol	1177	C <sub>10</sub> H <sub>18</sub> O	0.1	0.1
19	Methyl chavicol	1196	C <sub>10</sub> H <sub>12</sub> O	37.2	56.7
20	Geraniol	1253	C <sub>10</sub> H <sub>18</sub> O	0.1	–
21	$\alpha$ -Cubebene	1351	C <sub>15</sub> H <sub>24</sub>	0.1	0.8
22	Eugenol	1359	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	0.3	1
23	$\alpha$ -Copaene	1377	C <sub>15</sub> H <sub>24</sub>	0.4	0.2
24	$\beta$ -Bourbonene	1388	C <sub>15</sub> H <sub>24</sub>	–	0.1
25	$\beta$ -Cubebene	1388	C <sub>15</sub> H <sub>24</sub>	–	0.5
26	$\beta$ -Elemene	1391	C <sub>15</sub> H <sub>24</sub>	2.1	0.7
27	Methyl eugenol	1404	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	0.2	0.6
28	$\alpha$ -Cedrene	1412	C <sub>15</sub> H <sub>24</sub>	0.1	–
29	$\alpha$ -(Z)-Bergamotene	1413	C <sub>15</sub> H <sub>24</sub>	1.8	2.6
30	(E)-Caryophyllene	1419	C <sub>15</sub> H <sub>24</sub>	1.4	1
31	$\alpha$ -Guaiene	1440	C <sub>15</sub> H <sub>24</sub>	5.4	0.4
32	Aromadendrene	1441	C <sub>15</sub> H <sub>24</sub>	0.3	0.1
33	(E)- $\beta$ -Farnesene	1457	C <sub>15</sub> H <sub>24</sub>	1.7	1.6
34	Germacrene D	1485	C <sub>15</sub> H <sub>24</sub>	6.1	2.7
35	$\beta$ -Selinene	1490	C <sub>15</sub> H <sub>24</sub>	0.2	–
36	$\alpha$ -Zingiberene	1494	C <sub>15</sub> H <sub>24</sub>	0.3	–
37	Bicyclogermacrene	1500	C <sub>15</sub> H <sub>24</sub>	1.8	1.4
38	$\alpha$ -Bulnesene	1510	C <sub>15</sub> H <sub>24</sub>	–	1.2
39	$\gamma$ -Cadinene	1514	C <sub>15</sub> H <sub>24</sub>	2.5	2
40	$\delta$ -Cadinene	1523	C <sub>15</sub> H <sub>24</sub>	0.7	0.1
41	(Z)-Nerolidol	1533	C <sub>15</sub> H <sub>26</sub> O	0.2	0.1
42	$\alpha$ -Cadinene	1539	C <sub>15</sub> H <sub>24</sub>	0.1	0.1
43	(Z)-Calamenene	1540	C <sub>15</sub> H <sub>22</sub>	–	0.4
44	Spathulenol	1578	C <sub>15</sub> H <sub>24</sub> O	1.3	–

TABLE I. Continued

No.	Compound	RI	Molecular formula	Peak area, %	
				Inflorescence	Leaves
45	Caryophyllene oxide	1583	C <sub>15</sub> H <sub>24</sub> O	0.1	0.1
46	Alloaromadendrene	1641	C <sub>15</sub> H <sub>24</sub> O	–	0.1
47	$\beta$ -Eudesmol	1651	C <sub>15</sub> H <sub>26</sub> O	0.3	0.1
48	$\alpha$ -Cadinol	1654	C <sub>15</sub> H <sub>26</sub> O	6.1	3
49	$\alpha$ -Bisabolol	1686	C <sub>15</sub> H <sub>26</sub> O	0.3	0.1
50	Phytol	1943	C <sub>20</sub> H <sub>40</sub> O	0.1	0.1
	Total	–	–	98.8	99.9

Comparison of the volatile constituents of hydroponically grown *O. basilicum* with data published on the oil composition of this plant from wild habitats and field and open-air grown plants shows that there are some qualitative and quantitative discrepancy and/or similarity between the essential oil profiles of different plant production systems.<sup>10–14,18–27</sup> Moreover, there was a significant difference between the chemical profile of our present and previous work on field grown basil plants, when menthone and estragole were identified as the principal volatile oil components.<sup>9</sup>

TABLE II. Main classes and subclasses of hydroponically grown *Ocimum basilicum* L. essential oil constituents

Class and subclass of compounds	Content, %	
	Inflorescence	Leaves
Monoterpenoids	27.7	22.1
Monoterpene hydrocarbons	2.5	3.2
Oxygenated monoterpenes	25.2	18.9
Sesquiterpenoids	33.3	19.4
Sesquiterpene hydrocarbons	25	15.9
Oxygenated sesquiterpenes	8.3	3.5
Phenylpropanoids	37.7	58.3
Total identified	98.8	99.9

Overall, regarding the major essential oil components of the present study and reports of other scientists from elsewhere, it seems that the hydroponic production system has potential application for basil production. Furthermore, the acceptable volatile oil content along with volatile oil richness in methyl chavicol and linalool make the studied oil worthy of consideration in commercial markets. The minor chemical variations from different plant origins and production systems seems to be due to the impact of divergent climatological and geographical conditions (light quality and quantity, soil characteristics, water and nutrient (subspecies, natural hybridization and chemovariety), diverse growing conditions (wild habitats, common greenhouse production and different soil-less culture sys-

tems) as well as agronomic parameters (fertilization, irrigation regime and weed control). Those different variables dependently modify the assimilation capacity and hence interactive relationship between primary and secondary metabolism of aromatic plants in favor of the biosynthesis and accumulation of distinct volatile components.

#### CONCLUSIONS

The essential oil content and composition of hydroponically grown *O. basilicum* L. were comparable with those of previously reported wild and cultivated plants. In conclusion, the hydroponics system can be a promising production method for the yearly growth of this phenylpropane- and monoterpene-rich plant in order to satisfy the high demands of the pharmaceutical, fragrance and food industries.

#### ИЗВОД

#### САСТАВ ЕТАРСКОГ УЉА ЦВАСТИ И ЛИСТОВА БОСИЉКА ГАЈЕНОГ ХИДРОПОНИЧНО

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Одређиван је састав етарског уља листа и цвасти хидропонично гајеног босиљка *Ocimum basilicum* L. методом дестилације воденом паром и GC/EI-MS. Идентификовано је педесет састојака који су чинили укупно 98,8 % садржаја уља цвасти, односно 99,9 % уља листа. Фенилпропаноиди (37,7 % уља цвасти и 58,3 % уља листа) чине доминантну класу једињења, а затим следе сесквитерпени (33,3 и 19,4 %) и монотерпени (27,7 и 22,1 %). Од монотерпена, једињења са кисеоником су главна поткласа (25,2 и 18,9 %). Угљоводоници (25,0 и 15,9 %) су чинили главну поткласу сесквитерпенских једињења. Метил-чавикол, дериват фенилпропана, (37,2 и 56,7 %) основни је састојак оба уља, чинећи 38,0 % уља цвасти и 57,0 % уља листа. Линалол (21,1 и 13,1 %),  $\alpha$ -кадинол (6,1 и 3,0 %), гермакрен D (6,1 и 2,7 %) и 1,8-цинеол (2,4 и 3,5 %) су, такође, међу основним састојцима уља. Значајна је квантитативна, али не и квалитативна разлика између етарских уља два биљна органа. Састав етарског уља хидропонично гајеног босиљка *O. basilicum* L. је сличан саставу уља биљке гајене на земљи, што може бити од значаја за фармацеутску и прехранбену индустрију.

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