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Diversity in Chemical Composition and Yield of Essential oil from Three Ecotypes of Sweet Basil (*Ocimum basilicum* L.) in Iran

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ABSTRACT: Sweet basil (*Ocimum basilicum* L.) belong to the family Lamiaceae. The aim of this study was to identification of the components of sweet basil cultivated in Iran. The study carries out in Isfahan provinces, Central of Iran, on 2014. The essential oils of samples were obtained by hydro-distillation, and analyzed using GC/MS. The essential oil yields were obtained from the aerial of *O. basilicum*, 0.68, 0.71 and 0.96 ml / 100 g dry matter identified in Baghe-Bahadoran, Shahreza and Falavarjan province, respectively. In total, 30, 32 and 38compounds were identified in Baghe-Bahadoran, Shahreza and Falavarjan province, respectively. The major constituents of the oil Baghe-Bahadoranwere methyl chavicol (58.35%), neral (11.64%), linalool (9.34%) and 1,8-cineole (4.13%).The major constituents of the oil Shahreza were methyl chavicol (45.82%), linalool (17.31%), neral (13.21%), 1,8-cineole (5.73%), geranial (5.84%) and the major constituents of the oil Falavarjan were methyl chavicol (62.69%), geranial (7.36%), linalool (6.91%), β-caryophyllene (4.68%) and neral (4.67%).

Keywords: *Ocimum basilicum* L, chemical composition, methyl chavicol.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) belong to the family Lamiaceae is an annual, herbaceous, white to purple flowering plant, 20-60 cm tall, that originated in Iran and India. The genus Ocimum includes 50-150 species and subspecies (Labra et *al.,* 2004), distributed throughout the tropical regions of Asia, Africa and Central and South America (Mozaffarian, 2008). *O. basilicum* (2n = 48), is an important medicinal plant and culinary herb which is used in treatment of headaches, diarrhea, coughs, warts, worms and kidney malfunctions (Bais *et al.,* 2002; Rai *et al.,* 2004).

Secondary metabolites from Ocimum species possess exceptional biological activity and have antioxidant (Kwee and Niemeyer, 2011) and antimicrobial (Annand *et al.,* 2011), bactericidal (Haniff *et al.,* 2011), repellent (Nerioet al., 2010), anticonvulsant (Freire *et al.*, 2006), chemo preventive and radioprotective effects (Gajula *et al.,* 2009).

The essential oil from the aerial parts of *O. basilicum* consists of a wide and varyingarray of chemical constituents, depending on variations in chemotypes, leaf and flower colors, aroma, and origin of the plants (Chalchat and Ozcan, 2008; Carovic-Stanko *et al.,* 2010). Methyl chavicol, methyl cinnamate, methyl eugenol, citral, and linalool are generally the main chemotypes in sweet basil. Investigations (Sajjadi, 2006) on the chemical composition of the essential oil of basil, however, have demonstrated considerable variability. Golparvar *et al.* (2015) reported that phytochemical variations were not only found among samples of different regions but also among samples of the same region with different altitude reflecting the effect of environment on essential oil components. For example, Ghasemi Pirbalouti (2014) reported that the major constituents of the *O. basilicum* essential oil from the aerial of Purple landrace were methyl chavicol or estragol (63.32%) and linalool (7.96%). The main compositions of the essential oil from the aerial of Green landrace were methyl chavicol (31.82%), geranial (24.60%) and neral (22.65%). Sajjadi (2006) identified 20 constituents in the volatile oil of O. basilicumcv. Purple collected at full flowering which the main constituents were methyl chavicol (52.4%), linalool (20.1%), epi-α-cadinol (5.9%) and trans-α-bergamotene (5.2%) and 12 components in the volatile oil of O. basilicumcv. green collected at full flowering which methyl chavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%) were the major components. Reported by Telci *et al.* (2006) identified seven different chemotypes in 18 basil landraces from Turkey and majority were characterized by high linalool contents. Also, linalool was found as dominant constituent in essential oils in nine basil accessions from Italy (Labra *et al.,* 2004). Reported (Keita *et al.,* 2000) that the oil of *O. basilicum* contained linalool (69%), eugenol (10%), (E)-α-bergamotene (3%) and thymol (2%). The aim of this study was to diversity in chemical composition and yield of essential oil from three ecotypes of basil (*Ocimum basilicum* L.) in Iran.

MATERIAL AND METHODS

The aerial parts of basil (*Ocimum basilicum* L.) including "Green" were collected at before flowering from three ecotypes (Baghe-Bahadoran, Shahreza and Falavarjan province) at Isfahan, Southwest Iranon 2014 (Table 1).

Table 1: Geographical and environmental conditions collected from *O. basilicum* in geographic different.

Site no	Locality	Province	Latitude	Longitude	Altitude (m asl[1])
1	Baghe-Bahadoran	Isfahan	32°22'28.6" N	51°11'06.8" E	1589
2	Shahreza	Isfahan	32°00'41.3"N	51°52'12.4" E	1572
3	Falavarjan	Isfahan	32°28'02.3" N	51°28'01.4" E	1612

A. Essential oil extraction

The essential oils were extracted from 100 g of ground tissue in 1 L of water contained in a 2 L flask and heated by heating jacket at 100°C for 3h in a Clevenger-type apparatus, according to procedures outlined in the British Pharmacopoeia. The collected essential oil was dried over anhydrous sodium sulfate and stored at 4°C ± 1°C until analyzed.

B. Identification of the oil components

Compositions of the essential oils were determined by GC-MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m × 0.25 mm, 0.25 ?m film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20°C at 50°C for 4 min and programmed to 280°C at a rate of 5°C /min, and kept 20°C constant at 280°C for 5 min, at split mode. The injector temperature was at 20°C at 280°C. Transfer 20 line temperatures 280°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450. Retention indices were calculated for all components using a homologous series of n-alkanes (C5-C24) injected under conditions used with the oil samples. Identification of the essential oil components was accomplished based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/Chem Station data system) (Adams 2007).

RESULT AND DISCUSSION

The essential oils extracted from the aerial parts of *Ocimum basilicum* L. produced a clear, yellow liquid. The essential oil yields were obtained from the aerial of *O. basilicum*, 0.68, 0.71 and 0.96 ml / 100 g dry matter identified in Baghe-Bahadoran, Shahreza and Falavarjan province, respectively (Table 2).

The chemical constituents identified by GC-MS, are presented in Table 2. GC-MS analyses resulted in *O. basilicum* essential oil, 30, 32 and 38compounds were identified in Baghe-Bahadoran, Shahreza and Falavarjan province, respectively. The oil of Baghe-Bahadoran components corresponding to 99.24% and the major constituents of the oil Baghe-Bahadoran were methyl chavicol (58.35%), neral (11.64%), linalool (9.34%) and 1,8-cineole (4.13%).The oil of Shahreza components corresponding to 98.49% and the major constituents of the oil Shahreza were methyl chavicol (45.82%), linalool (17.31%), neral (13.21%), 1,8-cineole (5.73%) and geranial (5.84%). The oil of Falavarjan components corresponding to 98.99% and the major constituents of the oil Falavarjan were methyl chavicol (62.69%), geranial (7.36%), linalool (6.91%), β-caryophyllene (4.68%) and neral (4.67%).

Monoterpenes are a large and diverse class of volatile C_{10} isoprenoids that are the major constituents of many plant essential oils and resins. These natural products play important chemoecological roles in the interactions of plants with their environments (Hallahan, 2000). Great variations in the essential oil content of *O. basilicum* across geographic regions might be attributed to variable agroclimatic conditions and different agronomic techniques for cultivating (Hussain *et al.*, 2008). Wesolowska *et al.* (2012)reported that the major constituents of the *O. basilicum* essential oil from Station in Doluje near Szczecin. The following sweet basil cultivars were'Thai Siam', 'Bolloso Napoletano' and 'A Foglie di Lattuga.

The main components found in the oil 'Thai Siam' were linalool (24.60 and 36.60% in 2010 and 2011, respectively), (E)-methyl cinnamate (18.73 and 21.90%) and methyl chavicol (5.57 and 7.50%). The main components found in the oil' Bolloso Napoletano' were linalool (41.09and 47.75%), methyl chavicol (14.34 and 20.21%) and 1,8-cineole (7.21 and 10.23%). The main components found in the oil' A Foglie di Lattuga' were linalool (37.51 and 48.65%), methyl chavicol (13.41 and18.55%) and 1,8-cineole (7.72 and 12.59%). The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing. In addition, for maximum oil production, long days and high light intensities are required during the maturation period (Salehi *et al.*, 2014; Abedi *et al.*, 2015).

Report that there was a higher content of linalool in young leaves (Werker *et al.*, 1993). In *O. basilicum* from Bangladesh, linalool and geraniol are reported as the main components (Mondello *et al.*, 2002). In the oils, obtained from aerial parts of *O. basilicum* grown in Colombia and Bulgaria, linalool and methyl cinnamate are reported as major components of volatile oils respectively (Jirovetz and Buchbauer, 2001; Vina and Murillo, 2003).

Table 2: Chemical composition of essential oils three ecotypes of *Ocimum basilicum* L.

			% GC peak area		
Row	**Compound**	**RI**	**Baghe-Bahadoran**	**Shahreza**	**Falavarjan**
1	α-Thujene	926	0.12	tr	0.31
2	α-Pinene	935	0.41	0.24	0.17
3	Camphene	950	-	-	tr
4	Sabinene	975	tr	-	0.21
5	β-Pinene	977	-	0.41	0.19
6	β-Myrcene	994	0.34	0.71	0.14
7	α -Terpinene	1017	-	tr	-
8	p-Cymene	1025	0.13	0.24	-
9	Limonene	1029	0.37	0.18	0.26
10	1,8-Cineole	1034	4.13	5.73	3.51
11	*Trans*-β-Ocimene	1048	0.13	1.59	0.97
12	γ-Terpinene	1063	-	0.42	tr
13	Cis-sabinene hydrate	1074	tr	-	-
14	Fenchone	1089	1.21	0.96	0.84
15	Linalool	1104	9.34	17.31	6.91
16	Camphor	1146	tr	-	-
17	Borneol	1167	0.62	0.21	-
18	Terpinen-4-ol	1181	0.16	tr	0.15
19	α-Terpineol	1195	-	-	tr
20	Methyl chavicol (estragole)	1203	58.35	45.82	62.69
21	Octanol acetate	1216	tr	-	-
22	Nerol	1228	1.53	-	0.35
23	Neral	1243	11.64	13.21	4.67
24	Chavicol	1267	tr	-	-
25	Geraniol	1253	-	tr	0.34
26	Geranial	1274	3.68	5.84	7.36
27	Thymol	1285	-	-	0.11
28	Bornyl acetate	1289	0.31	0.12	-
29	Eugenol	1363	1.35	0.98	0.21
30	α -Copaene	1376	0.31	0.82	0.12
31	β-Cubebene	1384	0.12	tr	-
32	*Trans*-methyl cinnamate	1387	-	-	tr
33	β-elemene	1398	-	-	tr
34	Methyl eugenol	1412	0.16	0.37	0.18
35	β-Caryophyllene	1419	2.68	1.83	4.68
36	*Trans*-α-Bergamotene	1437	-	-	0.14
37	α-Guaiene	1442	-	tr	-
38	α-Humulene	1455	1.35	0.62	0.35
39	Germacrene-D	1482	0.38	0.41	0.98
40	Bicyclogermacrene	1496	-	tr	0.25
41	γ-Cadinene	1514	-	-	tr
42	*Trans*-α-bisabolene	1544	-	0.28	1.41
43	Spathulenol	1569	-	-	0.27
44	Caryophyllene oxide	1578	tr	tr	0.62
45	Veridiflorol	1583	-	-	0.21
46	1,10-di-*epi*-cubenol	1616	-	-	tr
47	*Epi*-α-cadinol	1643	-	-	0.11
48	β-Eudesmol	1652	-	0.12	0.21
49	α -Cadinol	1657	0.35	-	-
50	α -Bisabolol	1691	-	tr	-
	Total		99.24	98.49	98.99
	Essential oil yield (%)		0.68	0.71	0.96

RI: Retention indices determined on HP-5MS capillary column.tr, trace (< 0.1%).

Linalool and methyl eugenol are the main components of the essential oils of *O. basilicum* cultivated in Mali (Chalchat *et al.,* 1999) and Guinea (Keita *et al.,* 2000). In *O. basilicum* landraces from Turkey, the essential oil yields were from 0.4 to 1.5% (Bozin *et al.,* 2006; Telci *et al.,* 2006) while the yield of essential oil from *O. basilicum* grown in Serbia and Montenegro has been reported to be 0.37% (Klaus *et al.,* 2008).

CONCLUSION

In conclusion, the results obtained in our study indicated that the major components of the oil of *O. basilicum* collected from three ecotypes were methyl chavicolorestragole, linalool, 1,8-cineole, neral. Differences in the essential oils of studied *O. basilicum* could be attributed to genetic, chemotype, distinct environmental and climatic conditions.

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