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Original scientific paper

DIVERSITY IN CHEMICAL COMPOSITION AND YIELD OF ESSENTIAL OIL FROM TWO IRANIAN LANDRACES OF SWEET BASIL

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Ocimum basilicum L. belongs to the family Lamiaceae is an herb that is extensively cultivated in some countries. Areal parts, especially leaves of sweet basil are widely used to enhance the flavour of foods such as salads, pasta, tomato products, vegetables, pizza, meat, soups, marine foods, confectioneries and other products. Essential oil yield and chemical components of two Iranian landraces of sweet basil including "Purple" and "Green" grown south-central of Iran (Isfahan province) were investigated. The hydro-distillated oils were analyzed by GC–MS. The oil yields were obtained from the aerial of Purple with 0.56 ml / 100 g dry matter and the aerial of Green with 0.48 ml / 100 g dry matter. Results indicated significant differences ($p < 0.01$) among the aerial for the main constituents in the essential oil from two Iranian landraces of sweet basil. The major constituents of the 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%). Genarlly, a comparison of our results with the previous reports suggests differences in the essential oil compositions and oil yield of the plant material could be attributed to genetic diversity in two Iranian landraces of sweet basil.

Key words: genetic diversity, geranial and ,neral essential oil, methyl chavicol, *Ocimum basilicum* L.

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INTRODUCTION

Swtte basil (*Ocimum basilicum* L.) belong to tha family Lamiaceae is an annual, herbaceous, white to purple flowering plant, 20–60 cm tall, that originated in Iran and India (ÖZCAN *et al*. 2005; CHALCHAT & ÖZCAN, 2008) and has become a major essential oil cultured commercially in some countries. Sweet basil is a popular culinary herb and its essential oil has been used for many years to flavour foods, as an ingredient of dental and oral health care products and in fragrances (LACHOWICZ *et al*. 1998). The areil parts, espeically leaves of sweet basil are widely used to enhance the flavor of foods such as salads, pasta, tomato products, vegetables, pizza, meat, soups, marine foods, confectioneries and often products (ÖZCAN & CHALCHAT, 2002; GHASEMI PIRBALOUTI *et al*. 2013a). In Iranian traditional medicine, the areil parts of the plant are perceived as a carminative, galactogogue, stomachic, and antispasmodic (SAJJADI, 2006).

The essential oil from the areial parts of *O. basilicum* 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 & ÖZCAN, 2008; CAROVIĆ–STANKO *et al*. 2010). Methyl chavicol, methyl cinnamate, methyl eugenol, citral, and linalool are generally the main chemotypes in sweet basil. Investigations (SAJJADI, 2006; CAROVIĆ–STANKO *et al*. 2010) on the chemical composition of the essential oil of basil, however, have demonstrated considerable variability.

Various researchers reported that essential oil yield and its components in medicinal and aromatic plants in general is primarily related to their genetic (SHAFIE *et al*. 2009), climate, edaphic, elevation and topography (POUROHIT & VYAS, 2004; LOZIENE & VENSKUTONIS, 2005; RAHIMMALEK *et al*. 2009; GHASEMI PIRBALOUTI *et al*. 2013a,b,c), and their interaction (BASU *et al*. 2009). Recent findings indicated that some of the medicinal plant characteristics can be affected by genetic and ecological factors, including precipitation, temperature, plant competition, and nitrogen content in the soil (GHASEMI PIRBALOUTI *et al*. 2013b).

The main goal of this study was determine the variation of chemical composition and yield of the essential oils from the aerial parts of two Iranian landraces of sweet basil.

MATERIALS AND METHODS

Plant material

The aerial parts of two Iranian landraces of sweet basil including “Purple” and “Green” were collected at before flowering from Nazhvan farm lands at Isfahan, Southwest Iran (32° 38′ N and 51° 32′ E) about 1601 m above sea level. Plant identities were confirmed by Mr. Shrimardi and voucher specimen (IAUSHK–81G and IAUSHK–81P) have been placed in the Herbarium of Research Center of Medicinal Plants, Shahrekord Branch, I.A.U. Iran. A total of three replicate samples from each of the plants were gathered.

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 3 h 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 until analyzed.

Identification of the oil components

Compositions of the essential oils were determined by GC–MS. The GC analysis was done using an Agilent Technologies 7890 GC equipped with a HP-5MS 5% capillary column (30.00 m x 0.25 mm, 0.25 µm film thicknesses). The carrier gas was helium at a flow of 0.8 mL/min. Initial column temperature was 60 °C and programmed to increase at 4 °C/min to 280 °C. The split ratio was 40:1 and the injector temperature was set at 300 °C. The purity of helium gas was 99.999%. Essential oil samples (0.1 µL) were injected manually. The GC-MS analysis was done on the Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70 eV with a mass range from *m/z* 50–550. Retention indices were calculated for all components using a homologous series of *n*-alkanes (C_5-C_{24}) 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/ChemStation data system) (ADAMS, 2007).

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 $p \leq 0.05$ level.

RESULTS AN DISCUSSION

Essential oil yield

The essential oils extracted from the aerial parts of two Iranian landraces of basil produced a clear, yellow liquid. A significant difference ($p \leq 0.05$) in oil yields was obtained from the aerial parts of two Iranian landraces of basil. The essential oil yields were obtained from the aerial of Purple landrace with 0.56 ml / 100 g dry matter and the aerial of Green landrace with 0.48 ml / 100 g dry matter (Table. 1). An earlier report by SAJJADI (2006) indicated the oil yield of the aerial parts of *O. basilicum* cv. purple and *O. basilicum* cv. green collected at full flowering from the same region were 0.2% and 0.5% (v/w) respectively. TAHSILI *et al*. (2010) measured the oil yield in the aerial parts of *O. basilicum* collected from Alborz province, Iran, as 0.75% (v/w) before the flowering. The differences in the essential oil yield among the plant samples could be attributed to genetic diversity.

Chemical composition

The chemical constituents identified by GC-MS, are presented in Table 1 and Fig 1. In total, 38 and 28 components were determined in essential oils of Purple and Green landraces of basil, respectively that represented 95% of the oils (Table 1). The major constituents of the essential oil from the aerial of Purple landrace were methyl chavicol or estragol (63.32±6.0%) and linalool (7.96±0.1%). The main compositions of the essential oil from the aerial of Green landrace were methyl chavicol (31.82±5.4%), geranial (24.60±1.1%) and neral (22.65±0.7%). Similarly, SAJJADI (2006) identified 20 constituents in the volatile oil of *O. basilicum* cv. 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. basilicum* cv. 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. A

comparison of our results with the previous report by SAJJADI (2006) suggests few differences in the volatile composition of the plant material could be attributed to the growth and cultivation conditions of the plant, to the methods of extraction and to the harvesting time.

Table 1 Chemical compositions of essential oils of two basil landraces

Row	Compounds	RI	% GC peak area		*ANOVA*
			Purple	Green	
1	*α*-Thujene	928	0.17	0.14	
2	*α*-Pinene	935	0.19	0.42	
3	Camphene	949	0.12	-	
4	Sabinene	973	0.15	-	
5	Octen 3 ol	977	-	0.68	
6	*β*-Pinene	978	0.56	-	
7	Heptanol <2,6-dimethyl-2>	987	-	0.98	
8	*β*-Myrcene	991	0.66	0.25	
9	*α*-Terpipene	1021	0.09	-	
10	Limonene	1027	0.66	0.44	
11	1,8-Cineole	1030	3.49±0.8a	0.41±0.4b	$p < 0.01$
12	*β*-Ocimene <Z>	1035	0.20	-	
13	*β*-Ocimene <E>	1046	3.13±0.6a	0.04±0.1b	$p < 0.01$
14	□-Terpinene	1056	0.23	-	
15	Fenchone	1087	1.20	1.02	
16	Linalool	1098	**7.96±0.1a**	0.19±0.1b	$p < 0.01$
17	Camphor	1141	0.94	-	
18	Borneol	1162	0.27	-	
19	Menthol	1169	0.32	0.64	
20	Terpinene-4-ol	1173	0.55	-	
21	*α*-Terpineol	1189	0.31	-	
22	Methyl chavicol	1201	**63.32±6.0a**	**31.82±5.4b**	$p < 0.01$
23	Nerol	1227	-	1.00	
24	Pulegone	1234	-	0.27	
25	Neral	1242	0.28±0.4b	**22.65±0.7a**	$p < 0.01$
26	Geraniol	1253	-	0.26	
27	Geranial	1272	0.32±0.4b	**24.60±1.1a**	$p < 0.01$
28	Thymol	1285	0.51	0.29	
29	Carvacrol	1297	0.37	-	
30	*α*-Copaene	1370	0.06	0.12	
31	*β*-Cubebene	1384	-	0.17	
32	*β*-Elemene	1386	0.69	-	

Table 1 Continued

Row	Compounds	RI	% GC peak area		*ANOVA*
			Purple	Green	
33	Methyl eugenol	1399	0.7	0.51	
34	*β*-Caryophyllene	1407	1.40±0.6b	2.75±0.4a	$p < 0.01$
35	*α*-Bergamotene <Z>	1430	1.07	-	
36	*α*-Guaiene	1433	0.17	-	
37	*α*-Humulene	1446	0.72	2.04	
38	*β*-Farnesene <Z>	1451	0.56	0.15	
39	Germacrene-D	1474	1.27	0.96	
40	Bicyclogermacrene	1489	0.94	-	
41	□-Cadinene	1507	0.24	-	
42	*α*-Bisabolene <E>	1538	-	1.96	
43	Spathulenol	1569	-	0.08	
44	Caryophyllene oxide	1574	0.08	0.39	
45	Veridiflorol	1582	0.07	-	
46	*α*-Cadinol < *Epi*->	1633	1.44	-	
	Total		95.32	95.22	
	Essential oil yield		0.56±0.31a	0.48±0.02b	$p \leq 0.05$

[a]RI: Retention indices determined on HP-5MS capillary column.

Results indicated significant differences ($p < 0.01$) among the aerial for the main constituents in the essential oil from two Iranian landraces of sweet basil. The variability of the main constituents contents in the essential oils obtained from two Iranian landraces of basil attributed mainly to different genotypes and chemotypes (LOZIENE & VENSKUTONIS, 2005; RAHIMMALEK *et al*. 2009).

The volatile compounds from the purple landrace of basil could be grouped in the following main chemical groups: phenylpropanoids (64.01%), oxygenated monoterpenes (16.50%), sesquiterpenes hydrocarbons (7.08%), monoterpene hydrocarbons (6.15%) and oxygenated sesquiterpenes (1.59%), while in the green landrace the chemical groups could be grouped into oxygenated monoterpenes (51.34%), phenylpropanoids (32.33%), sesquiterpenes hydrocarbons (8.16%), monoterpene hydrocarbons (1.33%) and oxygenated sesquiterpenes (0.40%). Varition in the chemical composition of the studied landraces could be attributed to genetic (genus, species, sub species, and ecotype), and / or chemotype (GHASEMI PIRBALOUTI *et al.,* 2013b,c).

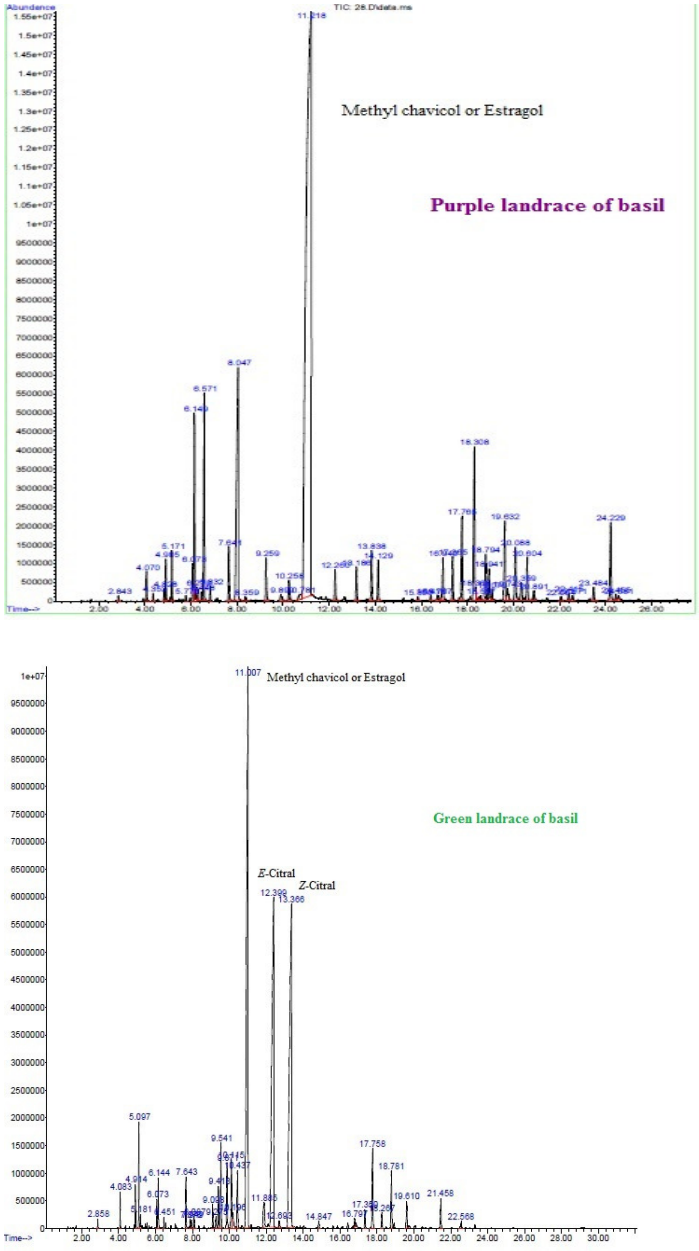

Fig 1. The chromatograms found in dried basil samples (for peak identification see Table 1).

CONCLUSION

The results of this study provide data on variation of phytochemical characteristics of the essential oils from two Iranian landraces of basil. The present study indicates the essential oil components of two Iranian landraces of basil vary with genotype and chemotypes. Phenylpropanoids and oxygenated monoterpenes were the main constituents of the essential oil of the aerial perts of the studied landraces of sweet basil. Results of current study indicate that methyl chavicol or estragol and linalool for the purple landrace and methyl chavicol, geranial and neral for the purple landrace are the main constituents of the essential oils. Generally, differences in the essential oil yield and its components among the studied landraces of basil could be attributed to genetic diversity.

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DIVERZITET HEMIJSKOG SADRŽAJA I PRINOSA ESENCIJALNOG ULJA DVE IRANSKE DIVLJE VRSTE BOSILJKA

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Izvod

Ocimum basilicum L., bosiljak, pripada familiji *Lamiaceae* i intezivno se gaji u nekim državam.. Svi delovi biljke .posebno listovi se koriste da se poboljša miris hrane kao što su salate, proizvodi od krompira, paradaiza, povrće, meso, supe, morska hrana. Vršena su ispitivanja prinosa esencijalnog ulja i njegove hemijske komponente kod divljih vrsta "Ljubičasti " i "Zeleni" koji se gaje u južnim i centralnim delovima Irana. Hidro-destilovana ulja su analizirana primenom GC–MS. Prinos ulja u arealu u kome se gaji lubičasti bosijlak je bio 0.56 ml / 100 g suve materije a u arealu gajenja zelenog bosiljka 0.48 ml / 100 g suve supstance. Reszultati pokazuju značajne razlike ($p < 0.01$) između areala u glavnim konstituentima esencijalnog ulja ispitivanih divljih vrsta. Generalno, poređenjem dobijenih rezultata sa ranije objavljenim ukazuje da su razlike u prinosu i kompoziciji esencijalnih ulja posledica genetičke divergentnosti ispitivanih divljih vrsta iranskog bosiljka.

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