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Phytochemical diversity of fennel landraces from various growth types and origins

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Abstract. The presence of certain secondary metabolites in fennel essential oil is the cause of its pharmacological and flavoring properties. In this study phytochemical diversity including essential oil content and compositions of 26 fennel landraces from various growth types and geographical originations were assessed. Essential oil content of the fennel landraces varied from 1.1 to 4.8%, with late and medium maturities showing higher essential oil contents than early maturities. According to the Gas Chromatography Mass Spectrometry (GCMS) results, the main essential oil components were trans-anethole (1.2–88.4%), methyl chavicol (0.2–59.1%), fenchone (1.1–14.7%) and limonene (5.3–15.7%). According to the clustering results, it was noticed that all the fennel landraces originated from arid climates were trans-anethole chemotype with an average concentration of 76%. The early and late maturity fennels from humid climates were methyl chavicol chemotype with an average concentration of 54%. The late maturities from humid and moderate climates were fenchone chemotypes with 12% concentration, and finally the early and late maturities from semi-arid climates were limonene chemotype with 12% concentration. Our results confirm that climate is a major evolutionary determining factor on the phytochemical diversity of fennel landraces.

Key words: Fennel, Essential oil, GCMS, Chemotype.

INTRODUCTION

The oldest evidence referring to usage of herbal medicines and spices traces back to sixty thousands years ago in Shanidar cave in Kurdistan (Lietava, 1992). Fennel (*Foeniculum vulgare* Mill.) belongs to the Apiaceae family. It is an essential-oil-producing aromatic plant and one of the oldest herbs at the global level. Bitter fennel subspecies (*F. Vulgare* Mill. *Vulgare*), possessing appealing flavor and beneficial medicinal effects and is cultivated for source subspecies for the fennel derived drugs (Hornok, 1992). Bitter fennel, hereafter named just as fennel is native to the Mediterranean areas and also has been naturalized in many other regions (Hornok, 1992; Guillen & Manzanos, 1994).

In human nutrition, every part of this plant including seeds, foliage and roots can be used in different ways (Barros et al., 2010; Edoardo et al., 2010). Fennel seed with its spicy odor and burning sweet taste has a special usage in condiments, perfumes, and industrial liqueurs as a flavoring reagent (Barros et al., 2010; Edoardo et al., 2010; Diao et al., 2014). From the aspect of medical care, the most famous usage of fennel is as an effective anti-colic that's even more effective than chemical drugs (Piccaglia & Marotti, 2001; Alexandrovich et al., 2003). Several studies have shown fennel herbal drugs have valuable antioxidant, anti-cancer, anti-inflammatory, antibacterial, and antifungal properties (Elagayyar et al., 2001; Choi & Hwang, 2004; Lucinewton et al., 2005; Singh et al., 2006; Anwar et al., 2009; El-Awadi & Esmat, 2010; Hamdy Roby et al., 2013; Diao et al., 2014; Ouariachi et al., 2014; Oliveira et al., 2015; Upadhyay, 2015); and several commercial pharmaceuticals are formulated based on fennel essential oil (Elagayyar et al., 2001; Edoardo et al., 2010). In livestock industries, the most significant improvement in a chick's body weight and feed efficiency was obtained by a diet enriched by fennel seed (Mohammed & Abbas, 2009; Teixeira et al., 2013). Naturally essential oil has an important role in attracting insects for pollination (Bowes & Zheljazkov, 2005), however fennel essential oil can be used as a natural pesticide in field and greenhouse crops, and as an anti-mold in foods products (Isman et al., 2011; Regnault Roger et al., 2012; Ebadollahi et al., 2014; Tabrizi et al., 2014). This subject is an important issue in organic food production, and also in cockroach and mosquito control in the human environment (Isman et al., 2011). Some other applications of fennel essential oil are in aromatherapy and massage centers as plant oil (Bowes & Zheljazkov, 2005; Upadhyay, 2015) and in metal industries as a corrosion inhibitor (Lahhit et al., 2011). All the aforementioned pharmacological features and flavoring properties of fennel are due to the presence of certain secondary metabolites in the essential oil; therefore fennel quality is associated with its essential oil content and compositions (Upadhyay, 2015).

More than 90% of fennel essential oil is stored in reproductive organs, especially in seed secretory channels (Akgiil & Bayrak, 1988; Stefanini et al., 2006). According to different studies, the range of fennel seed essential oil content has been reported between 0.6–6% (Bowes & Zheljazkov, 2005; Zahid et al., 2008; Najdoska et al., 2010; Shojaiefar et al., 2015). Essential oil content in the leaves of Iranian fennels had been investigated and ranges from 0.6–2% (Rahimmalek et al., 2014). The main essential oil compositions of fennel are monoterpenes including trans-anethole, methyl chavicol, fenchone and limonene (Singh et al., 2006; Radulovic & Blagojevic, 2010; Shahat et al., 2011). In the study by Rahimmalek et al. (2014), the major essential oil compositions of Iranian fennels leaves were trans-anethole, limonene, and fenchone, with no observation of any methyl chavicol.

Trans-anethole accounts for the anise taste serves as a pleasing aroma in food and perfumes, and as an effective anti-flatulence agent in herbal medicines. Methyl chavicol provides a sweet taste and is mainly used in the perfume industry, while fenchone is responsible for the bitter and spicy taste of fennel, and acts as a real antidepressant. Finally, limonene has well-established chemo-preventive activity against cancers and is also used in resins and solvents (Guilled & Manzanons, 1996; Miraldi, 1999; Pank et al., 2003; Singh et al., 2006; Sun, 2007; Anwar et al., 2009; He & Huang, 2011; Acimovic et al., 2015). According to previous studies about essential oil compositions of bitter fennel, trans-anethole content ranges from 0.1–78%, methyl chavicol 0.1–81.2%, fenchone 1–18.7%, and limonene 1–22%. Trans-anethole has been mentioned as the most dominant component in bitter fennel essential oil (Piccaglia & Marotti, 2001;

Stefanini et al., 2006; Gulfraz et al., 2008; Aprotosoaie et al., 2010; Edoardo et al., 2010; Shahat et al., 2011; Aprotosoaie et al., 2013; Hamdy Roby et al., 2013; Moghtader, 2013; Acimovic et al., 2015; Upadhyay, 2015). Bitter fennel landraces from Asia have higher trans-anethole content rather than from Africa and Europe (Ouariachi et al., 2014), and European fennels have higher methyl chavicol content (Bilia et al., 2002; Edoardo et al., 2010; Basaglia et al., 2014). According to a review by Edoardo et al. (2010), studies about essential oil composition of bitter fennel from Italy have revealed three chemotypes including trans-anethole type, methyl chavicol type, and methyl chavicol/trans-anethole type. According to Ozcan & Chalchat (2006), *F. vulgar Piperitum*, another subspecies of fennel, is mostly methyl chavicol chemotype.

The total world fennel seed production in 2014 was approximately 845 thousand tons (FAO, 2014). In certain industries, the demand for fennel essential oil is rapidly rising; this necessitates the need to develop specific chemotypes. Therefore, fennel needs more attention from researchers, and the first step is to gain the knowledge of present diversity and phytochemical pattern in order to find chemotypes that can guide appropriate selection schemes and breeding programs (Judzentiene & Mockute, 2010).

Among the effective factors on essential oil quality, genetic backgrounds and growth environmental condition are the most important ones (Bowes & Zheljazkov, 2005; Curado et al., 2006; Medina Holguın et al., 2007; Anwar, 2009; Telci et al., 2009; Najdoska et al., 2010; Rahimmalek et al., 2014; Elhassan & Hussein Ayoub, 2014). It is a well-known fact that growth environmental condition can only offer a condition for minor and temporarily changes in essential oil, while the effect of genetic background is major and permanently which is important to us (Bach, 1995; Nemeth, 2005; Medina Holguın et al., 2007; Aprotosoaie et al., 2010). The genetic background of any genome relates to its evolutionary adaptions to the environment in which the ancestors have lived (Amos & Harwood, 1998; Heywood, 2002; Murray et al., 2004; Ramırez Valiente et al., 2009); and that is why botanists describe a healthy gene pool as the one possesses the ability to respond to environmental changes (Amos & Harwood, 1998). Every population experiences a unique environment; hence, a special diversity pattern is expected in each. Fennel populations are widely distributed throughout Iran and have occupied different habitats with diverse ecological conditions. Due to the local environment adaptation, it is assumed each region has its own specific fennel landrace. This subject has been proved morphologically and phenologically (Bahmani et al., 2012a; Bahmani et al., 2015; Shojaiefar et al., 2015), genetically (Bahmani et al., 2012b; Bahmani et al., 2013; Shojaiefar et al., 2015) and cytogenetically (Sheidai et al., 2007).

Identifying the phytochemical pattern in fennel landraces will help to gain accessibility to the desired chemotypes and decrease the threats of genetic erosion. Iranian fennel landraces have never been comprehensively studied for essential oil content and components, so we decided to do this. The aims of this study were: 1. Evaluation of essential oil content and compositions in different Iranian fennel landraces and 2. Identification of phytochemical diversity pattern and specific chemotypes.

MATERIALS AND METHODS

Plant material

In this study, fennel seeds (*Foeniculum vulgare* Mill.) were provided by seed bank of Aburaihan College / Tehran University. This seed bank was founded by the first two authors of this research in 2010 through gathering ecotypes from different parts of Iran. These ecotypes were separately propagated in the research field of Aburaihan College located in Pakdasht possessing annual averages of 175 mm precipitation and 16.9 °C temperature (Table 1). The same place of growth for the landraces means their differences are only due to genetic dissimilarities. For this study in 2015, we selected 26 fennel landraces from that fennel seed bank, based on two criteria: 1. Landraces should include all growth types (early, medium and late maturity) and 2. Their origins should be different (Table and Fig. 1).

Table 1. Geography profile of originations of the 26 fennels landraces

No	Landraces	Growth type (Day to maturity)	Altitude (m)	Longitude	Latitude	Climate (Koppen climate classification)
1	Sari	Late maturity	23	53 0 E	36 33 N	Semi humid/moderate
2	Kaleibar	(240)	1,105	47 1 E	38 52 N	Semi arid/moderate
3	Hajiabad		931	55 55 E	28 19 N	Arid/warm
4	Qazvin		1,278	50 0 E	36 15 N	Semi arid/moderate
5	Chahestan		27	56 22 E	27 13 N	Arid/warm
6	Meshkinshahr	Medium	1,578	47 40 E	38 23 N	Semi arid/cold
7	Fozveh	maturity	1,650	51 26 E	32 36 N	Arid/moderate
8	Kohin	(180)	1,668	49 67 E	36 36 N	Semi arid/cold
9	Kashan		972	51 27 E	33 59 N	Arid/warm
10	Khash		1405	61 12 E	28 13 N	Arid/warm
11	Moqhan		31	47 55 E	39 39 N	arid/moderate
12	Ardabil		1,332	48 17 E	38 15 N	Semi arid/cold
13	Khalkhal		1,769	48 31 E	37 38 N	Semi arid/cold
14	Damavand		2,000	52 15 E	35 43 N	Semi arid/cold
15	Marvdasht		1,502	52 83 E	29 80 N	Arid/warm
16	Oromie	Early maturity	1,332	45 4 E	37 33 N	Semi arid/cold
17	Fasa	(120)	1,288	53 41 E	28 58 N	Arid/warm
18	Shiraz		1,488	52 32 E	29 36 N	Arid/warm
19	Sabzevar		987	57 43 E	36 12 N	Arid/cold
20	Mahalat		1,775	50 45 E	33 91 N	Semi arid/cold
21	Incheboron		460	55 57 E	37 53 N	Arid/moderate
22	Sanandaj		1,350	47 0 E	35 20 N	Semi arid/cold
23	Rafsanjan		1,580	55 54 E	30 25 N	Arid/warm
24	Hashtgerd		1,426	50 43 E	35 65 N	Arid/cold
25	Yazd		1,230	54 36 E	31 89 N	Arid/warm
26	Bajestan		1,265	58 17 E	34 51 N	Arid/moderate
	Pakdasht		1,025	51 67 E	35 47 N	Arid / moderate

Table 1 continues

Pakdasht	Spring	Summer	Fall
Average precipitation (mm)	35.6	4.5	61.8
Average temperature (°C)	19.5	30.3	10.6

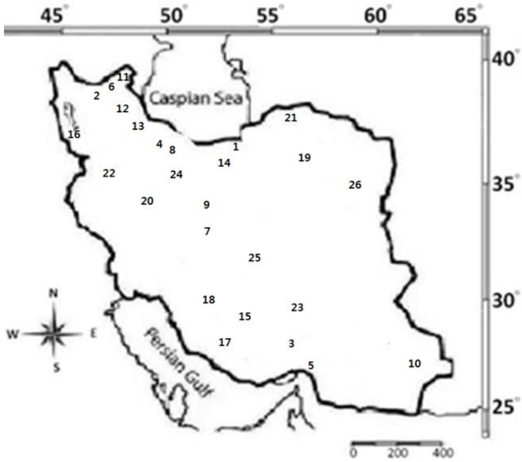

Figure 1. Locations of the 26 Iranian fennel landraces on map (modified from www.enchantedlearning.com).

Essential oil extraction

The essential oil was extracted from the ripened seeds by water distillation in a Clevenger apparatus (Boyadzhieva & Angelov, 2014). After extraction, the essential oils were stored in glass vials in 5 °C until the essential oil compositions analysis by GCMS.

Essential oils composition

In the 26 landraces, determination of essential oils composition were conducted by GC-MS analysis (a combined analytical method to identify different substances within a sample); Varian CP-3800 GC (Gas Chromatography) coupled with Varian 4000 (Ion trap) MS (Mass Spectrometry) equipped with a capillary VF-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at the constant flow of 1.0 ml min^{-1}; split ratio, 1/50. Mass spectra were taken at 70Ev and Mass range was from m/z 35–400 a.m.u. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 3 °C min^{-1}, and held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. The essential oil compositions were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a VF-5 column under the same chromatographic conditions. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature. For quantification purposes, relative area percentages obtained by FID were used without the use of correction factors.

Statistical analysis

In this study, essential oil component yield was calculated by this formula: essential oil content × essential oil component concentration / 100. The graphs were drawn by Excel 2010 and clustering analysis of the landraces was done by SPSS 18.

RESULTS AND DISCUSSION

Essential oil content

The amounts of essential oil content (%) of the landraces were shown in Table 2, and according to that, essential oil content in the studied fennel landraces ranged from 1.1 to 4.8% (cc 100 gr^{-1} seed).

Average essential oil content in early, medium and late maturity fennel landraces were 1.9% ± 0.2, 3.1% ± 0.2, and 3.5% ± 0.4 respectively. Late and medium maturity fennels had the highest amounts of essential oil content. These two were originated from areas with longer and proper growth condition: a circumstance that evolutionary has led them to higher vegetative growth, and consequently higher secondary metabolites production. The same result was obtained from a study by Zahid et al. (2008) saying late maturity fennel landraces with higher vegetative growth have higher essential oil content. The essential oil content in seeds of Iranian fennels is higher than that in their leaves (Rahimmalek et al., 2014). The essential oil contents that we got in this study were almost similar to the previous study on fennel (Bowes & Zheljazkov, 2005; Najdoska et al., 2010; Rahimmalek et al., 2014; Shojaiefar et al., 2015).

Table 2. Essential oil content (%) of the 26 Iranian fennel landraces

Growth type	No	Landraces	Essential oil content (%)	Mean (%)
Late maturity	1	Sari	4.8	3.5 ± 0.46
	2	Kaleibar	4.3	
	3	Haji abad	2.3	
	4	Qazvin	3.5	
	5	Chahestan	2.7	
Medium maturity	6	Meshkinshahr	2.9	3.1 ± 0.25
	7	Fozveh	3	
	8	Kohin	4.1	
	9	Kashan	4	
	10	Khash	3.3	
	11	Moqhan	2.5	
	12	Ardabil	2.2	
	13	Khalkhal	2.4	
	14	Damavand	2.1	
	15	Marvdasht	4.2	
Early maturity	16	Oromie	2.8	2 ± 0.21
	17	Fasa	2	
	18	Shiraz	2.6	
	19	Sabzevar	3.2	
	20	Mahalat	1.4	
	21	Inche boron	2.4	
	22	Sanandaj	2.5	
	23	Rafsanjan	1.1	
	24	Hasht gerd	1.4	
	25	Yazd	1.3	
	26	Bajestan	1.6	

Essential oil compositions

According to Table 3, the results of essential oil GC-MS analysis showed there is a huge qualitative and quantitative difference among Iranian fennel landraces. This diversity has a significant impact on the pharmaceutical and flavoring effects of the essential oil (Aprotosoaie et al., 2013). Based on Table 3, generally, 28 components in our fennel's essential oils were identified. Trans-anethole, methyl chavicol, fenchone, and limonene were the main components existing in all the landraces, constituting 76.7–99.4% of the total values. Additional essential oil components existing in all the landraces were α-Pinene (averagely 1.13%), Sabinene (0.54%), Myrcene (0.79%), α-Phellandrene (0.72%), Ortho-Cymene (0.46%) and γ-Terpinene (0.96%) (Table 3).

According to Table 3, in the Iranian fennel landraces, trans-anethole ranged from 1.2 to 88.4%; methyl chavicol from 0.2 to 59.1%; fenchone from 1.1 to 14.7%; and limonene from 5.3 to 15.7%. This diversity among Iranian fennels is because of genetic dissimilarities (polymorphism) which is in turn due to the diversity of their geographical origin (Bowes & Zheljazkov, 2005; Curado et al., 2006; Medina Holguın et al., 2007; Anwar, 2009; Telci et al., 2009; Najdoska et al., 2010; Kahrizi et al., 2011; Elhassan & Hussein Ayoub, 2014; Rahimmalek et al., 2014). Clustering analysis based on the major essential oil components including trans-anethole, methyl chavicol, fenchone and limonene for the landraces has been done.

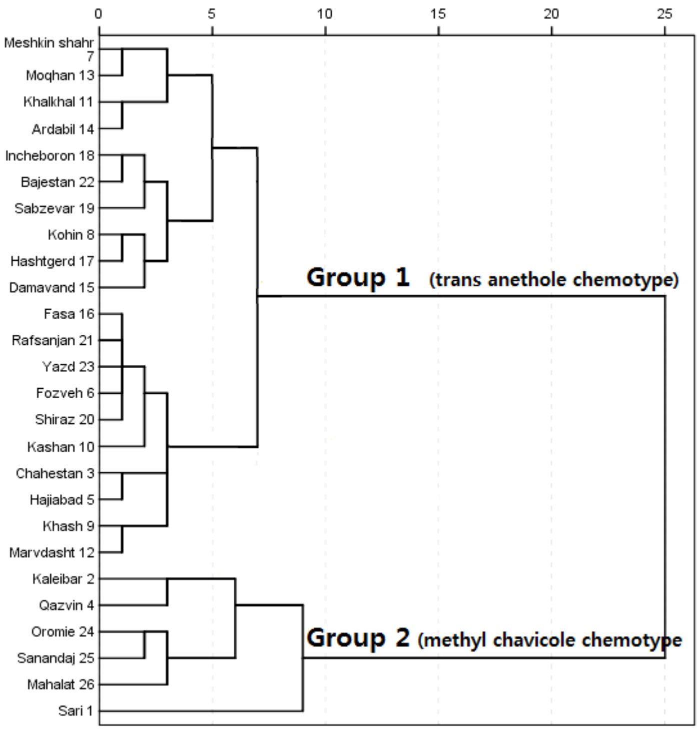

Figure 2. Dendrogram of the 26 fennel landraces with the most important essential oil components using Euclidean coefficient and WARD methods.

Table 3. Essential oil compositions (%) of the 26 Iranian fennel landraces

Composition	RI	Sari	Kaleibar	Chahestan	Qazvin	Hajiabad	Fozveh	Meshkinshahr	Kohin	Khash
α –Thujene	927	0.04	-	0.01	-	0.02	-	-	-	-
α-Pinene	936	1.34	1.02	0.91	1.56	0.6	0.86	0.51	0.21	0.15
Camphene	954	0.2	0.14	0.08	-	-	0.06	0.02	0.08	-
Sabinene	976	0.2	0.12	0.2	1.08	0.71	0.14	0.11	0.14	0.01
β-Pinene	983	0.07	0.04	0.03	0.13	-	0.02	0.02	0.05	-
Myrcene	991	0.97	0.29	0.33	0.88	1.56	0.34	0.17	0.1	0.06
α-Phellandrene	1,011	9.4	0.27	0.08	0.94	0.36	0.08	0.06	0.1	0.01
Ortho -Cymene	1,028	0.83	0.09	0.19	0.9	0.75	0.11	0.15	0.12	0.01
β-Ocimene	1,032	-	-	-	1.2	0.7	-	-	-	-
Limonene	**1,034**	**5.62**	**13**	**5.8**	**11.01**	**5.3**	**9.91**	**8.21**	**9.12**	**6.72**
β-Phellandrene	1,037	0.79	0.09	0.44	0.42	-	-	0.02	0.11	-
Delta.3-Carene	1,037	0.41	-	-	-	-	0.67	0.31	-	0.13
α-Ocimene	1,044	-	-	-	-	-	-	-	-	-
γ-Terpinene	1,062	0.89	1.07	0.87	0.9	0.9	0.89	0.37	0.02	0.09
Fenchone	**1,096**	**14.74**	**9.15**	**6.4**	**9.03**	**5.98**	**3.11**	**3.9**	**4.38**	**4.06**
allo-ocimene	1,128	-	-	-	0.19	-	-	-	-	-
β-Terpinolene	1,144	0.12	0.1	0.04	-	-	0.04	0.03	0.06	-
Camphor	1,154	0.27	0.17	0.07	0.8	0.6	0.11	-	0.016	-
Methyl Chavicol	**1,205**	**55.09**	**59.1**	**1.39**	**52.55**	**1.6**	**3.17**	**17.12**	**10.14**	**0.22**
Fenchyl acetate (endo)	1,222	0.78	0.1	0.11	-	-	0.03	-	0.07	-
Fenchyl Acetate (exo)	1,236	6.17	0.4	0.4	0.45	0.26	0.09	0.02	-	0.03
Cis-Anethole	1,253	-	-	0.03	-	-	-	-	-	0.01
Iso-bornyl acetate	1,287	0.13	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**1.24**	**14.7**	**82.3**	**16.73**	**80.5**	**79.9**	**68.71**	**75.04**	**88.45**
alpha.-Cubebene	1,345	-	-	-	1.3	-	-	-	-	-
Carvotanacetone	1,347	-	-	-	-	-	-	-	-	-
α-Copaene	1,381	0.04	-	0.03	-	-	0.01	-	0.03	-
Germacrene-D	1,487	0.44	0.08	0.27	-	-	0.15	0.16	0.17	-

Table 3 continues

Composition	RI	Kashan	Khalkhal	Marvdasht	Moqhan	Ardabil	Damavand	Fasa	Hashtgerd	Yazd
α –Thujene	927	-	-	-	-	-	-	-	-	-
α-Pinene	936	1.29	3.03	1.03	1.47	1.45	2.01	1.65	2.64	0.2
Camphene	954	0.02	0.28	0.21	-	0.27	0.5	0.2	0.39	-
Sabinene	976	0.12	0.69	0.78	0.59	0.46	0.5	0.44	0.6	1.02
β-Pinene	983	0.04	0.23	0.16	-	0.16	0.24	-	0.16	-
Myrcene	991	0.3	1.44	0.4	0.9	1.34	0.45	1.12	2.03	0.26
α-Phellandrene	1,011	0.11	0.33	0.37	0.66	0.34	0.65	0.31	0.5	0.95
Ortho -Cymene	1,028	0.12	0.87	0.93	1.06	0.7	0.39	0.22	0.35	0.13
β-Ocimene	1,032	-	1.05	1.02	1.33	1.4	1.6	1.8	3.77	0.34
Limonene	**1,034**	**12.4**	**6.54**	**6.8**	**8.1**	**6.62**	**8.5**	**7.41**	**8.14**	**7.22**
β-Phellandrene	1,037	-	1.17	0.62	-	-	0.99	0.57	0.44	-
Delta.3-Carene	1,037	0.81	0.32	-	-	0.21	-	-	-	-
α-Ocimene	1,044	-	-	-	-	-	0.24	-	0.16	0.4
γ-Terpinene	1,062	1.11	1.83	1.51	0.91	1.31	1.84	1.1	1.48	0.98
Fenchone	**1,096**	**2.49**	**4.09**	**4.02**	**3.31**	**4.13**	**4.64**	**1.16**	**4.14**	**2.28**
allo-ocimene	1,128	-	-	0.17	0.24	-	0.31	-	0.22	0.35
β-Terpinolene	1,144	-	-	-	-	-	-	-	-	-
Camphor	1,154	-	0.6	0.76	0.53	0.91	1.13	0.78	0.97	1.58
Methyl Chavicol	**1,205**	**2.5**	**14.35**	**0.45**	**17.28**	**16.15**	**9.59**	**3.4**	**9.97**	**4.1**
Fenchyl acetate (endo)	1,222	0.19	-	-	-	-	0.62	-	-	-
Fenchyl Acetate (exo)	1,236	0.3	1.56	0.45	0.81	0.56	0.9	1.02	-	0.32
Cis-Anethole	1,253	0.05	-	-	-	0.5	-	-	-	-
Iso-bornyl acetate	1,287	-	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**77.63**	**60.39**	**86.17**	**68.74**	**62.28**	**70.26**	**78.43**	**74.28**	**79.25**
alpha.-Cubebene	1,345	-	1.29	0.74	-	1.1	1.65	-	1.43	0.28
Carvotanacetone	1,347	-	-	-	-	-	-	-	-	-
α-Copaene	1,381	0.04	-	0.31	-	-	-	0.37	-	0.08
Germacrene-D	1,487	0.43	-	-	-	-	-	-	-	-

Table 3 continues

Composition	RI	Sabzevar	Shiraz	Rafsanjan	Bajestan	Oromie	Sanandaj	Mahalat	Incheboron
α –Thujene	927	-	-	-	-	-	-	-	-
α-Pinene	936	0.68	0.66	0.9	0.8	1.79	1.04	0.5	1.08
Camphene	954	0.15	0.02	-	0.98	0.43	-	0.024	-
Sabinene	976	0.34	0.43	0.45	2.36	1.26	0.23	0.283	0.76
β-Pinene	983	0.02	0.02	-	-	0.28	0.03	0.053	0.54
Myrcene	991	1.27	0.52	1.23	1.56	1.62	0.28	0.25	0.87
α-Phellandrene	1,011	0.08	0.18	0.66	0.7	0.8	0.12	0.11	0.62
Ortho -Cymene	1,028	0.03	0.02	1.65	0.32	0.37	0.04	0.15	1.52
β-Ocimene	1,032	-	-	1.85	0.86	0.84	-	-	1.48
Limonene	**1,034**	**5.5**	**10.59**	**8.52**	**7.32**	**12.1**	**15.71**	**11.52**	**6.3**
β-Phellandrene	1,037	-	0.65	-	1.01	1.23	-	0.15	0.94
Delta.3-Carene	1,037	0.97	0.51	-	-	-	-	0.48	-
α-Ocimene	1,044	-	-	0.23	0.3	-	-	-	-
γ-Terpinene	1,062	0.08	0.98	0.89	1.9	1.4	0.18	0.47	1.2
Fenchone	**1,096**	**2**	**1.39**	**1.54**	**1.28**	**1.3**	**1.22**	**2.09**	**2.85**
allo-ocimene	1,128	-	-	-	0.53	0.31	-	-	-
β-Terpinolene	1,144	0.02	0.11	-	-	-	-	-	-
Camphor	1,154	0.05	0.15	0.86	2.03	1.14	-	-	-
Methyl Chavicol	**1,205**	**3.4**	**3.5**	**2.49**	**3.12**	**52.27**	**54.98**	**50.89**	**5.71**
Fenchyl acetate (endo)	1,222	-	-	-	-	-	-	-	-
Fenchyl Acetate (exo)	1,236	0.04	0.26	0.26	1.64	1.18	0.03	0.048	0.5
Cis-Anethole	1,253	0.04	-	-	-	-	-	-	0.25
Iso-bornyl acetate	1,287	-	-	-	-	-	-	-	-
Trans-Anethole	**1,299**	**68.96**	**78.98**	**78.16**	**72.66**	**24.79**	**25.86**	**32.69**	**71.65**
alpha.-Cubebene	1,345	-	-	-	0.57	1.9	-	-	-
Carvotanacetone	1,347	-	-	-	-	-	-	-	3.41
α-Copaene	1,381	16.1	0.03	0.28	0.1	-	-	-	-
Germacrene-D	1,487	0.26	0.64	-	-	-	0.23	0.08	-

RI-retention indices relative to C6–C25 n-alkanes on the DB-5 column; t – trace < 0.1%.

As shown in Fig. 2, the 26 Iranian fennel landraces were divided into two groups: Group 1 as the trans-anethole chemotype (averagely 76%) and Group 2 as the methyl chavicol chemotype (averagely 54%). With a closer glance at Fig. 2, we noticed that group 1 includes all the fennel landraces originated from the arid areas of Iran (annual mean precipitation 225 mm) and group 2 includes all the fennel landraces originated from the humid areas of Iran (annual mean precipitation 585 mm). Also, group 1 has two sub-groups; the first sub-group (1-I) includes all landraces from the arid/cold area of southern Alborz (averagely 69%), and the second sub-group (1-II) includes all landraces from arid/warm areas of eastern Zagros (averagely 82%). Group 2 has three sub-groups; the first sub-group (2-I) includes late maturity landrace of Sari from northern Alborz which has a semi-humid/moderate climate in Iran. The second sub-group (2-II) includes early maturity landraces of Oromie, Sanandaj, and Mahalat from western Zagros which has a semi-arid/cold climate. The third sub-group (2-III) includes late maturity landraces of Kaleibar and Qazvin from the middle part of Alborz which has a semi-arid/moderate climate. The three sub-groups of group 2 have almost the same rate of methyl chavicol and the differences among them are related to fenchone and limonene content. Due to the fact that a big part of Iran has low precipitation, most of the Iranian fennels are trans-anethole chemotype. Iranian fennel landraces have been completely acclimatized to the area that they came from. According to Edoardo et al. (2010), Italian bitter fennels were divided into three chemotypes including trans-anethole type, methyl chavicol type, and methyl chavicol/trans-anethole type. In Fig. 3, the topographical features of Iran were shown.

According to Fig. 3, there are two big mountain chains in Iran: Alborz and Zagros Mountain Chains. The climatic features of Iran are the results of these two. Alborz Mountain Chain is like a wall that separates the northern side from the southern side of Iran from the northwest to the northeast of the country and blocks humid winds blowing inland from the Caspian Sea. It provides Iran with a humid northern side, with average annual precipitation of 1,000 mm, and an arid southern side, with average annual precipitation of 250 mm. Zagros Mountain Chain also acts like a wall, running from the northwest to the south of the country, and blocks humid winds blowing from the Mediterranean Sea. It also separates the western side from the eastern side of Iran, and provides Iran with a humid western part, averaging 600 mm precipitation annually, and an arid eastern part that averages 200 mm precipitation annually (Masodian, 2002). In Table 4, details of the 2 main groups and also climatical information of their origins were shown.

Figure 3. Zagros and Alborz mountain chains
(modified from www.worldatlas.com).

Table 4. The pattern of the content of essential oil composition of early, medium and late maturity fennels (climatic data from www.irimo.ir)

Group	Sub-group	Trans anethole (%)	Methyl chavicole (%)	Fenchone (%)	Limonene (%)
1	1-I	**69.29 ± 1.5**	10.68 ± 1.71	3.47 ± 0.34	7.43 ± 0.36
	1-II	**82.47 ± 0.14**	1.67 ± 0.41	4.07 ± 0.58	7.21 ± 0.71
2	2-I	1.24	**55.09**	**14.74**	5.62
	2-II	27.78 ± 2.47	**52.71 ± 1.2**	1.53 ± 0.27	**13.11 ± 1.31**
	2-III	15.71 ± 1.01	**55.82 ± 3.27**	**9.09 ± 0.06**	**12 ± 0.99**

Table 4 continues

Group	Sub-group	Origins	Climate	Annual Precipitation (mm) / air humidity (%) / temperature (°C)
1	1-I	Southern Alborz	Arid/cold	250 / 50 / 10
	1-II	Eastern Zagros	Arid/warm	200 / 45 / 20
2	2-I	Northern Alborz	Semi humid/moderate	900 / 75 / 16
	2-II	Western Zagros	Semi-arid/cold	450 / 60 / 10
	2-III	Middle Alborz	Semi-arid/moderate	400 / 55 / 15

The bold numbers show the highest values of essential oil compositions.

According to Table 4, the highest trans-anethole content was found in the landraces related to sub-group 1-I from eastern Zagros with arid/warm weather (200 mm precipitation and 20 °C temperature) and sub-group 1-II from southern Alborz with arid/cold weather (250 mm precipitation and 10 °C temperature). The evolutionarily determining factors in trans-anethole chemotypes development are firstly dryness and secondly hotness of weather in fennel origin areas. This finding is similar to what Rahimmalek et al. (2014) found out about the positive correlation of trans-anethole concentration in fennel leaves with a high temperature of its origins. We can name the fennels from eastern Zagros as 'the first-grade trans-anethole chemotypes' with 82% trans-anethole content, and those from southern Alborz as 'the second-grade trans-anethole chemotypes' with 69% trans-anethole content.

Apparently all of the medium maturity landraces were trans-anethole chemotype (group 1), while early and late maturities could be trans-anethole or methyl chavicol chemotypes. Rahimmalek et al. (2014) claimed that leaves of late maturity fennels have higher trans-anethole, however, in our study only seeds of those late maturity landraces from arid and not humid climate had high trans-anethole concentration.

Fennel seeds of trans-anethole chemotypes (group 1) are so useful to feed domestic animals. The trans-anethole, which can act as phytosterol, improves the final yield like egg / meat (Piccaglia & Marotti, 2001), so group 1 could be the selected chemotype to produce fennel seed to feed animals.

The highest methyl chavicol content was related to the late and early maturity landraces in group 2 including; northern Alborz (2-I), middle Alborz (2-III) and western Zagros (2-II) which are the most humid areas in the country (averagely 585 mm precipitation). It would be logical if we say humid weather evolutionarily has been the determining factor in methyl chavicol chemotype development. We can name the late maturity fennel landraces from northern and middle Alborz (groups 2-1 and 2-III) as 'the first grade methyl chavicol chemotypes' with 55% methyl chavicol concentration, and those early maturity landraces from western Zagros (group 2-II) as 'the second grade methyl chavicol chemotypes' with 52% methyl chavicol concentration (Table 4).

According to previous studies (Miraldi, 1999; Gross et al., 2009; Raal et al., 2011), there is a negative correlation between trans-anethole and methyl chavicol (which is the same result from our study). Gross et al. (2009) proposed that trans-anethole and methyl chavicol probably have a common biosynthetic precursor. They also reported the action of a bi-allelic gene in the biosynthesis of trans-anethole and methyl chavicol, with partial dominance for high methyl chavicol content. According to Molino (2000), methyl chavicol is produced exclusively through transformation (isomerization) of trans-anethole, but the reverse situation is also possible.

The highest fenchone content was found in the late maturity landrace from northern and middle Alborz (2-I and 2-III). It seems that late maturity and being originated from humid and moderate climate is a good marker for finding fenchone chemotype. We can name those fennels from northern Alborz as 'the first-grade fenchone chemotypes' with 15% fenchone concentration, and those from middle Alborz as 'the second-grade fenchone chemotypes' with 9% fenchone concentration (Table 4).

According to Miraldi (1999) fenchone chemotypes are originated from areas near to sea; in our study, the identified fenchone chemotypes from northern and middle Alborz are very close to the Caspian Sea which gains a lot of humidity and weather is moderate. We should consider that late fennel landraces from the south of Iran are also near to sea but have not been included in fenchone chemotype; the climate in the south of Iran is extremely arid and hot.

A high quality fennel essential oil has fewer than 10% methyl chavicol and fenchone which makes it taste sweet and proper for food industries (De Vincenzi et al., 2000; Bilia et al., 2002; Bowes & Zheljazkov, 2005; Zahid et al., 2008), based on that those landraces from arid areas of eastern Zagros and Southern Alborz (Group 1 in Table 4) are the best ones.

The highest limonene contents were related to landraces from western Zagros (2-II) and middle Alborz (2-III) averagely with 425 mm precipitation and 12.5 °C temperature. Apparently being originated from an area with normal annual precipitation and temperature is in favor of development of fennel limonene chemotype. We can logically name early maturity fennel landraces from western Zagros as 'the first-grade limonene chemotypes' with 13% limonene concentration, and those late maturity landraces from middle Alborz as 'the second-grade limonene chemotypes' with 12% limonene concentration (Table 4).

Positive correlation of methyl chavicol with limonene reported by Raal et al. (2011), was also confirmed that by our study (about group 2-II and 2-III in Table 4).

According to Oliveira et al. (2015) and Singh et al. (2006) those fennel landraces with high concentration of trans-anethole and/or limonene (group 1 and group 2-II) have a high potential of cytotoxic activity against fungi and also tumor cell lines, based on this Iranian fennels should be considered a promising source to develop specific antitumor source.

In fennel species, the content and compositions of essential oil in the same landrace in different years and even seasons are not majorly different (Bernath et al., 1996; Bowes & Zheljazkov, 2005; Stefanini et al., 2006; Aprotosoaie et al., 2010). In this study we assessed 26 fennel landraces from all over the country from all kinds of growth types and originations; so we can universalize these results for other similar geographies and also for fennel plants with different ages.

According to Tables 2 and 3, the landraces with the highest trans-anethole yield were medium maturity landraces (Marvdasht, Kashan, Khash and Fozveh 3 ± 0.2 cc 100 gr^{-1} seed) from eastern Zagros (group 1-II). Landraces with the highest methyl chavicol and fenchone yields were late maturity landraces (Sari, Kaleibar and Qazvin 2.3 ± 0.2 and 0.47 ± 0.11 cc 100 gr^{-1} seed respectively) from northern and middle Alborz (groups 2-I and 2-III). Landraces with the highest limonene yield (0.42 ± 0.3 cc 100 g^{-1} seed) were Kaleibar, Kashan, Sanandaj, Qazvin, Kohin and Oromie.

CONCLUSION

There is a good chance for Iranian fennels to be known and grown as a suitable source of fennel essential oil. Among Iranian fennels, all landraces originated form arid climates were trans-anethole chemotype with average concentration of 76%, those early and late maturity fennels from humid climates were methyl chavicol chemotype with average concentration of 54%, those late maturities from humid and moderate climate were fenchone chemotypes with 12% concentration, and those early and late maturities from semi-arid climates were limonene chemotype with 12% concentration. After finding the desired fennel chemotypes, it is necessary to optimize the environment that we should grow them because we must keep in mind that environmental condition has an effect on essential oil content and composition (Medina Holguın et al., 2007; Aprotosoaie et al., 2010). Our results confirmed that climate is a major evolutionary determining factor on the phytochemical diversity of fennel landraces. According to Piccaglia and Marotti (2001) in fennel landraces the origin latitude which majorly determines the climate has a significant effect on fennel essential oil; this subject also was proved in other plants (Nemeth, 2005). According to Rahimmalek et al. (2009), those yarrow genotypes related to the same chemotypes have been originated from similar climates. There is no direct explanation for these relationships, anyway, changes in regulating genes and enzymes under effects of water and temperature are probably the reason for that (Bach, 1995; Nemeth, 2005). The topic of fennel essential oil is getting more important especially when new usages of fennel essential oil are being found out. Regarding that it is worth to say that fennel essential oil could be extracted even from its callus (Bahreini et al., 2015). This subject opens a new door to those industries that use fennel essential oil and also a new method of enhanced solvent-free microwave extraction can offer numerous advantageous like short extraction time, less energy consumption and lower cost (Benmoussa et al., 2016).

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