Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 3, p. 401-407, 2015
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Quantitative and qualitative responses in chemical composition of three ecotypes of fennel (*Foeniculum vulgare* Mill.) cultivated in Iran climatic conditions

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Article published on March 28, 2015

Key words: chemical compositions, GC/MS, fennel, *Foeniculum vulgare* Mill.

Abstract

Fennel (*Foeniculum vulgare* Mill.), is a small genus of annual, biennial or perennial herbs and aromatic plant belonging to the family Apiaceae. The aim of this study was to identify of the chemical components of fennel cultivated in Iran climatic conditions. The seeds of *F. vulgare* were collected from three ecotypes (Kermanshah, Hamadan and Shahrekord) province in Iran, during 2014. The essential oil was extracted by a Clevenger approach and analyzed using GC/MS. In total, 28, 30 and 24 compounds were identified in seeds of fennel in Kermanshah, Hamadan and Shahrekord province, respectively. The major components in Kermanshah were; trans-anethole (63.21%), fenchone (9.2%), linalool (7.12%), *p*-cymene (4.13%), limonene (3.17%) and methyl chavicol (3.14%). The major components in Hamadan were; trans-anethole (70.2%), *p*-cymene (5.4%), linalool (5.41%), fenchone (4.6%) and methyl chavicol (4.21%). The major components in Shahrekord were; trans-anethole (61.5%), fenchone (11.5%), methyl chavicol (7.31%), limonene (6.7%), linalool (6.16%) and p-cymene (3.46%). The differences in the quantity or quality of the oils composition of the present and previous studies may be because of the chemotypes, phenological stage, drying conditions, mode of distillation and geographic and climatic factors.

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Introduction

Fennel (*Foeniculum vulgare* Mill.), is a small genus of annual, biennial or perennial herbs and aromatic plant belonging to the family Apiaceae. The herb was well-known to the ancient Egyptians, Romans, Indians, and Chinese. Fennel which is known as Razianeh in Persian and Bitter fennel, sweet fennel in English. Seeds are oblong to ovoid with 3–5 mm long and 1.5–2.0 mm broad. Thestylopodium persists on the fruit. The seeds are elongated and have strong ribs. Fennel which is diploid (2n=22) (Badgujar and Bandivdekar, 2014; Mozaffarian, 2008; Krishnamurthy, 2011; Diaaz-Maroto *et al.,* 2006). Two fennel varieties are of pharmaceutical importance, *F. vulgare* Mill, sub-species *vulgare* var. *dulce* (Mill) Thellung has (sweet fennel) and *F. vulgare* Mill, sub-species *vulgare* var. *vulgare* (piperitum) has (bitter fennel). The fennel plant originated in the southern Mediterranean region and through naturalization and cultivation it grows wild throughout the Northern, Eastern, and Western hemispheres, specifically in Asia, North America, and Europe. It is cultivated in fields and also grows wild (Rather *et al.,* 2012; Rechinger 1987). Fennel is used in various traditional systems of medicine like in the Ayurveda, Unani, Siddha, in the Indian, and Iranian traditional systems of alternative and balancing medicine (Rahimi and Ardekani, 2013). *F. vulgare* is used in many parts of the world for the treatment of a number of diseases, for example, abdominal pains, antiemetic, aperitif, arthritis, cancer, colic in children, conjunctivitis, constipation, depurative, diarrhea, dieresis, emmenagogue, fever, flatulence, gastralgia, gastritis, insomnia, irritable colon, kidney ailments, laxative, leucorrhoea, liver pain, mouth ulcer, and stomachache (Badgujar *et al.,* 2014; Rather *et al.,* 2012; Choi and Hwang 2004; Koppula and Kumar 2013). The family Apiaceae has more than 300 genera and 3000 species of the aromatics. Plants of this family can produce monoterpenes, sesquiterpenes, and phenyl components and related resins in their secretary ducts, roots, stem, leaf, flowers, seeds, and fruits (Sodeifian and Ansari, 2011). *F. vulgare* has been reported to contain 6.3% of moisture, 9.5% protein, 10% fat, 13.4% minerals, 18.5% fibre and 42.3% carbohydrates. The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C (Rather *et al.,* 2012). Volatile components of fennel seed extracts include trans-anethole, fenchone, methylchavicol, limonene, α-pinene, camphene, β-pinene, β-myrcene, α-phellandrene, 3-carene, camphor, and cisanethole (Ozcan *et al.,* 2003). Researchers have not covered the presence of high essential oil variation in fennel plants collected from various parts of different countries. Raal *et al.,* (2011) assessed the essential oil variations in commercial fennel seeds obtained from retail pharmacies in Estonia, Norway, Austria, and Moldova and from a spice shop in Turkey. In studies (Stefanini *et al.,* 2006) the main compinents of the oils of *Foeniculum vulgare* in Brazil were trans-anethole (78.25%) during the summer in dry seeds, limonene (42.30%) in spring in steams/leaves and fenchone (16.98%) in green seeds in autumn. Singh reported the main components of the oils of *Foeniculum vulgare* in India was trans-anethole (70.1%) but (Ozcan *et al.,* 2003) reported the main components of the fruit oils of *Foeniculum vulgare* was estragole (61.08%), fenchone (23.46%), limonene+ β-phellandrene + 1, 8-cineole (8.68%) and α-pinene (1.15%) (Singh *et al.,* 2006) and they concluded responses of this plant in chemical composition is variable in different ecotypes. The aim of this study was to identify of the chemical components of the essential oil from three ecotypes of Fennel (*Foeniculum vulgare* Mill.) cultivated in Iran.

Materials and methods

Plant materials

Seeds of (*Foeniculum vulgare* Mill.) were collected from annual plants of three ecotypes (Kermanshah, Hamadan and Shahrekord) province of Iran in during 2014 and were sown (Table 1,2).

Table 1. Collection site information, flowering time of fennel ecotypes in the present work.

Site no	Province	Latitude	Longitude	Elevation (masl)	T_{max} (°C)	T_{min} (°C)	Flowering time	Collected time
1	Kermanshah	34° 18 ′N	47° 03 ′E	1350	35.1	5.1	Late	September
2	Hamadan	34° 48 ′N	48° 31 ′E	1671	20.8	3.6	Very early	August
3	Shahrekord	32° 19 ′N	50° 51 ′E	2070	24.1	2.3	Very early	August

T_{max}, Maximum temperature (°C); T_{min}, Minimum temperature (°C).

Table 2. Some physical and chemical properties of soil collection site.

Site no	Province	PH	EC (ds/m)	Sand (%)	Silt (%)	Clay (%)	Total Nitrogen (%)	P (ppm)	K (ppm)
1	Kermanshah	7.35	2.26	46.14	35.16	18.16	0.54	41	171
2	Hamadan	6.54	1.12	35.21	52.15	12.64	0.68	69	214
3	Shahrekord	7.28	1.78	18.69	77.52	3.79	0.41	54	142

Essential oil extraction

Seeds of fennel accessions were collected and dried at room temperature (25 ± 5 °C). For each hydro-distillation turn, 50-60 g of samples was used. The round-bottom flask of Clevenger-type apparatus was used to extract essential oils. Four hundred ml distilled water was added and boiled for 5 hours. Then, the essential oil was collected in a container. The collected essential oil was dried over anhydrous sodium sulphate and stored at 4 °C until analyzed.

Identification of the oil components

Compositions of the essential oils were determined by GC–MS. The GC-MS analysis was performed using a Hewlett Packard 5973 with a fused silica capillary column 5% phenyl-poly-dimethyl-siloxane (DB-5MS 30 m x 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas (helium) flow rate was 1mL/min. 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 (Adams, 2007).

Results and discussion

Chemical composition

Qualitative and quantitative analysis of the essential oils volatile profile are listed in Table 3. In total, 28, 30 and 24 compounds were identified in seeds of fennel in Kermanshah, Hamadan and Shahrekord province, respectively. The major components in Kermanshah were; trans-anethole (63.21%), fenchone (9.2%), linalool (7.12%), *p*-cymene (4.13%), limonene (3.17%) and methyl chavicol (3.14%). The major components in Hamadan were; trans-anethole (70.2%), *p*-cymene (5.4%), linalool (5.41%), fenchone (4.6%) and methyl chavicol (4.21%). The major components in Shahrekord were; trans-anethole (61.5%), fenchone (11.5%), methyl chavicol (7.31%), limonene (6.7%), linalool (6.16%) and *p*-cymene (3.46%). (*Foeniculum vulgar* Mill.) of different origins contains almost the same components, and differences are found in the percentage of components. In their studies, cultivated plants from three ecotypes showed higher percentages of trans-anethole, fenchone, linalool, *p*-cymene, limonene and methyl chavicol. F. vulgare were rich in trans-anethole and higher percentages trans-anethole of Hamedan origins than the other origins (Table 3). The composition of medicinal plant can highly be affected by their secretary tissue condition and developmental stage (Gross *et al.,* 2002). In fennel oil, the major constituent is trans-anethole that might be variable in different stages of development (Gross *et al.,* 2002). Trans-anethole is derived from the general phenylpropanoid pathway, through which lignin, flavonoids and other phenylalanine-derived compounds are produced (Gross *et al.,* 2002). The chemical composition of (*F. vulgare*) has been studied by different researchers. For example, according to (Moghtader, 2013) the main constituents of the essential oil seeds of (*F. vulgare*) in Kerman province were trans-anethole (49.64%), fenchyl acetate (14.21%), estragole (8.67%), fenchone (6.37%)

and limonene (4.23%), also the main constituents of the essential oil flowers were trans-anethole (55.68%), Fenchyl acetate (11.56%), estragole (6.87%), fenchone (4.43%) and limonene (3.18%). In studies (Rahimmalek *et al.,* 2014) the leaf essential oil composition of 12 Iranian accessions of fennel collected from different geographical regions. The essential oil yield of fennel leaves ranged from 0.65% (Varamin accession) to 2.03% (Tabriz accession). Trans-anethole ranged from 41.19% in Shiravan to 56.6% in Shiraz accessions and had negative correlation with most of the constituents. In studies (Lahhit *et al.,* 2011) showed that the major components of (*F. vulgare*) from Morocco were limonene (20.8 %), β-pinene (17.8%), myrcene (15%) and fenchone (12.5%). In studies (El Ouariachi *et al.,* 2014) the major components of (*F. vulgare*) from Morocco were limonene (20.8%), β-pinene (17.8%), myrcene (15.0%) and fenchone (12.5%). The major compounds of Ground fennel (*F. vulgare* Mill.) seeds in Montenegro were trans-anethole (68.6 to 75.0%) and (62.0%), methylchavicol (5.09 to 9.10%) and (4.90%), fenchone (8.40 to 14.7%) and (20.3%) (Damjanovic *et al.,* 2005). In a study the chemical composition of seeds of *F. vulgare* Miller from two Spanish locations (Santander and Aranjuez) two different chemotypes have been found in the seeds, in one chemotypes methyl chavicol (54.9%), fenchone (24.6%) in the seeds collected in Santander; and in other chemotypes (E)-anethole (54.9 to 38.1%), fenchone (22.2 to 34.4%) in the seeds gathered in Aranjuez (Garcia-Jimenez *et al.,* 2000). In acomparison study of 13 fennel (*F. vulgare* Mill. var. *vulgare*) populations of different relatively stable and variable characteristics were distinguished from evaluating the correlation matrix of the morphological and chemical properties from two successive years and the medium strength correlation has been found only between the level of essential oil accumulation and the size of seeds (Bernath *et al.,* 1996). The oil obtained from air-dried seeds of *F. vulgare* of Turkish origin contained methyl chavicol (47.09%), limonene (29.07%), fenchone (13.43%), fenchyl acetate (1.95%), *cis*-β-ocimene (1.41%), α-pinene (1.22%), and myrcene (1.08%) as the main constituents (Bernath *et al.,* 1996). The most abundant classes of constituents of all four analyzed samples were the phenylpropanoids (69.5-85.5%) and monoterpenoids (11.7-26.9%). Another probable reason that might increase *trans*-anethole in fennel is the increase in the oil duct area, as observed in transversal cross sections in early stages of fennel development. Upon maturation, oleoresin is further accumulated due to increased duct volume as a result of duct elongation. Higher fruit development can lead to the lack of further metabolism and minimal volatilization, as indicated by apparent high lignification of the cells lining the oil ducts (Gross *et al.,* 2002). The dominant volatile metabolites of the schizocarps were fenchone (13.3-18.8%) and (E)-anethole (66.1-69.0%) (Radulovic and Blagojevic, 2010). According to (Msaada *et al.,* 2007) maturation stages play an important factor influencing essential oil composition, while suitable environmental and agricultural practices would also help in improving yield and quality. Despite records on essential oil composition of fennel fruit.

Table 3. Chemical composition of essential oil of (*Foeniculum vulgare* Mill.) from three different localities of Iran.

NO	Compound	RI	Percentage		
			Kermanshah	Hamedan	Shahrekord
1	α-Thujene	929	0.54	0.21	0.45
2	α-Pinene	935	0.35	0.25	0.12
3	α-Fenchene	950	0.1	0.12	-
4	Camphene	952	0.45	0.45	0.53
5	Sabinene	975	0.25	0.35	0.24
6	β- Pinene	980	-	0.21	0.18
7	β-Myrcene	991	0.51	0.14	0.57
8	Octanal	1002	-	0.13	-

NO	Compound	RI	Percentage		
			Kermanshah	Hamedan	Shahrekord
9	α-Phellandrene	1008	0.12	0.51	0.14
10	*P*- cymene	1025	4.13	5.4	3.46
11	Limonene	1031	3.17	1.45	6.7
12	β-Phellandrene	1032	0.31	0.32	0.13
13	1,8-Cineole	1035	0.62	0.12	0.14
14	(E)-β-Ocimene	1037	0.13	0.14	-
15	γ- Terpinene	1058	0.41	0.35	0.17
16	Terpinolene	1078	0.54	0.43	0.74
17	Fenchone	1089	9.2	4.6	11.5
18	Linalool	1098	7.12	5.41	6.16
19	endo-Fenchol	1116	0.21	-	0.17
20	exo-Fenchol	1121	0.13	-	0.25
21	Camphore	1148	1.13	1.24	0.15
22	α-Terpineol	1185	0.8	0.74	0.61
23	Methyl chavicol	1198	3.14	4.21	7.31
24	*p*-Anis aldehyde	1258	0.11	0.25	-
25	trans-Anethole	1288	63.21	70.2	61.5
26	Thymol	1290	-	0.16	-
27	α-Copaene	1371	0.18	0.21	0.42
28	*p*-Acetonylanisole	1380	0.12	0.21	-
29	β-Caryophyllene	1424	-	0.17	-
30	Germacrene D	1480	0.31	0.14	0.25
31	Spathulenol	1540	0.16	0.21	0.15
32	α-Cadinole	1564	0.14	0.12	-
	Tatal		97.59	98.45	95.73

RI, Retention indices determined on HP-5MS capillary column.

Conclusion

In conclusion, the results obtained in our study indicated that the major components of oil of *F. vulgare* collected from three ecotypes (Kermanshah, Hamadan and Shahrekord) province of Iran were trans-anethole, fenchone, linalool, *p*-cymene, limonene and methyl chavicol. The essential oils of *F. vulgare* collected from three ecotypes were rich in trans-anethole. Among the ecotypes, Hamadan had the highest acceptable *trans*-anethole content and it can be introduced as a good candidate for cultivation.

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