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Full Length Research Paper

Comparative survey on the essential oil composition from the seeds and flowers of *Foeniculum vulgare* Mill. from Kerman province

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This study was designed to examine the chemical composition of the essential oil of *Foeniculum vulgare* Mill. (apiaceae). The chemical composition of the essential oil from seeds and flowers of *F. vulgare* from Kerman province, Iran was obtained by the hydrodistillation method, analyzed by gas chromatography and gas chromatography mass spectrometry. Twenty four compounds, accounting for 99.75% of the total oil with 2.75% oil yield were identified in the essential oil of the seeds. The main constituents of the essential oil were *trans*-anethole (49.64%), Fenchyl acetate (14.21%), estragole (8.67%), fenchone (6.37%) and limonene (4.23%). Twenty six compounds, accounting for 98.28% of the total oil with 1.96% oil yield were identified in the essential oil of the flowers. The main constituents of the essential oil were *trans*-anethole (55.68%), Fenchyl acetate (11.56%), estragole (6.87%), fenchone (4.43%) and limonene (3.18%).

Key words: *Foeniculum vulgare* Mill., *trans*-anethole, essential oil composition, gas-chromatography/mass-spectrometry (GC/MS).

INTRODUCTION

Fennel is one of the precious medicinal plant that are widely used in pharmacy, perfume, cosmetic, hygienic and food industries (Zargari, 1995). Fennel has a long history of herbal use and is a commonly used as household remedy, being useful in the treatment of a variety of complaints, especially those of the digestive system. The seeds, leaves and roots can be used, but the seeds are most active medicinally and are the part normally used. An infusion is used in the treatment of indigestion, abdominal distension, stomach pains etc. An essential oil obtained from the seed is used in aromatherapy. Fennel contains anethole, which can explain some of its medical effects. The essential oil is bactericidal, carminative and stimulant (Huxley, 1992).

Fennel (*Foeniculum vulgare*), is a plant species in the genus *Foeniculum* (treated as the sole species in the genus by most botanists). It is a member of the family Apiaceae (formerly the Umbelliferae). It is a hardy, perennial, umbelliferous herb, with yellow flowers and feathery leaves. It is generally considered indigenous to the shores of the Mediterranean, but has become widely naturalized elsewhere (particularly, it seems, areas colonized by the Romans) and may now be found growing wild in many parts of the world, especially on dry soils near the sea-coast and on river-banks. Florence fennel or finocchio is a selection with a swollen, bulb-like stem base that is used as a vegetable. Fennel is used as a food plant by the larvae of some Lepidoptera species

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including the mouse moth and the anise swallowtail.

Fennel is an evergreen perennial herb. It is erect, glaucous green, and grows to heights of up to 2.5 m, with hollow stems. It is hardy to zone 5 and is not frost tender. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects. The plant is self-fertile. The leaves grow up to 40 cm long; they are finely dissected, with the ultimate segments filiform (threadlike), about 0.5 mm wide. (Its leaves are similar to those of dill, but thinner.) The flowers are produced in terminal compound umbels 5 to 15 cm wide, each umbel section having 20 to 50 tiny yellow flowers on short pedicels. The fruit is a dry seed from 4 to 10 mm long, half as wide or less, and grooved (Blamey and Grey-Wilson, 1989). On account of its carminative properties, fennel is chiefly used medicinally with purgatives to allay their side effects, and for this purpose forms one of the ingredients of the well-known compound liquorice powder.

The content of essential varies strongly (0.6 to 6%); fruits in the center of an umbel are generally greater, greener and stronger in fragrance. Time of harvest and climate are also important. The essential oil of *F. vulgare* plant has been studied in Iran and other countries but the chemical composition of the essential oil of *F. vulgare* grown in Kerman province is yet to be determined. In the present work we have analyzed the chemical composition of the seeds and the flowers of *F. vulgare* Mill. that grows in Kerman province in Iran and then the results were compared with various origins in other countries.

MATERIALS AND METHODS

Plant material collection and isolation of their essential oil

The seeds and flowers of *F. vulgare* plant were obtained from plants grown in a village in Kerman province, Iran at full flowering stage in August 2012. The samples were cleaned in shade condition to prevent volatility of the plant material constituents and to keep the natural color of the sample fixed. Then they were air-dried and were powdered using a milling machine and kept in a cool dry place until ready for extraction of the essential oil. Afterwards, essential oil was taken from 150 g of the powdered sample in hydrodistillation method with the help of Clevenger set for three hours. Following the sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields from seeds and flowers were calculated.

Analysis of essential oil

Gas chromatography (GC)

GC analysis was performed using a model HP-439 gas chromatograph equipped with column CP Sil. 5CB in 25 m length, internal diameter of 0.25 mm and film thickness 0.39 µm. Oven temperatures was from 60 to 220°C at a rate of 7°C slope per minute. Injector temperature was 280 centigrade and detector (FID) temperature was 270°C and carrier gas was helium.

Gas chromatography/mass spectrometry

In order to analyze and identify the combinations forming the essential oil, the Chromatograph gas set attached to a mass spectrometry, Model Hewlett Packard-5973 was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS in 60 m length, internal diameter of 0.25 mm and layer thickness of 0.25 mm, thermal program of oven (3 min) in 60°C, then 60 to 220°C with a 6°C slope per minute, then 3 min in 220°C, the temperature of place of injection 280°C, gas conveying helium, the speed of gas move 1.0 ml per minute, the ratio of fission 1 to 43, the rate of injection 0.1 ml, temperature of the reservoir of ionization 230°C, ionization mode EI, Ionization energy 70eV. The series of normal Alkans C8-C17 were also injected to the set under the same condition with that of essential oil injection to calculate restrictive index (RI) of components of essential oil. The restrictive index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 (DVD-ROM) existing in absolution software of gas-chromatography/mass-spectrometry (GC/MS) set and calculation of standard restrictive index in accordance with C_8-C_{17} Alkans and comparing them with the existing standard figurers in references (Adams, 2001).

RESULTS AND DISCUSSION

The identified combinations in essential oil, restrictive index (RI), and quantitative percentage of the compounds from seeds and flowers are listed in Table 1. The study of the analysis of *F. vulgare* Mill. essential oil under investigation showed that twenty four compounds, accounting for 99.75% of the total oil with 2.75% (v/w) oil yield were identified in the essential oil of the seeds. The main constituents of the essential oil were *trans*-anethole (49.64%), Fenchyl acetate (14.21%), estragole (8.67%), fenchone (6.37%) and limonene (4.23%) with 83.12% constituting the highest percentage of essential oil. Also from twenty six compounds, accounting for 98.28% of the total oil with 1.96% (v/w) oil yield were identified in the essential oil of the flowers. The main constituents of the essential oil were *trans*-anethole (55.68%), Fenchyl acetate (11.56%), estragole (6.87%), fenchone (4.43%) and limonene (3.18%) with 81.72% constituting the highest percentage of essential oil.

The quality and quantity of the materials forming *F. vulgare* Mill. essential oil had some differences and similarities with the cases reported in other regions. The studies of the ingredients of the essential oil of botanical populations with ecological and genetic differences can be of great importance in identifying the variety of essential oil inside the population of specie. It seems that the geographical origin of *F. vulgare* Mill. greatly influences the oil quality. The essential oil of *F. vulgare* Mill. plant has been widely studied in Iran and other countries but the chemical composition of the essential oil of *F. vulgare* grown in Kerman province is yet to be determined. In present study, results showed the major oil constituents of the seeds and the flowers of *F. vulgare*

Table 1. Combinations identified in the essential oil of *F. vulgare* Mill.

Compound name	(RI)	Seed (%)	Flower (%)
α-Thujene	926	1.21	0.68
α-Pinene	935	2.36	1.35
Camphene	957	0.28	-
Sabinene	978	0.78	1.49
β-Pinene	987	1.45	1.67
Myrcene	992	1.08	-
α-Phellandrene	998	0.24	0.45
P-Cymene	1025	-	1.65
Limonene	1035	4.23	3.18
1,8-Cineole	1038	1.37	0.47
(E)-β-Ocimene	1045	-	0.28
γ-Terpinene	1067	-	0.19
Fenchone	1079	6.37	4.43
Terpinolene	1085	0.46	-
Camphore	1138	0.96	-
α-Terpineol	1203	0.14	1.25
Estragole	1218	8.67	6.87
Dihydro carveol	1229	-	0.85
Trans-caveole	1235	-	0.24
Fenchyl acetate	1247	14.21	11.56
Cis-anethol	1252	3.67	2.24
Trans-cinnamaldehyde	1258	0.21	-
Trans-anethole	1269	49.64	55.68
Methyl acetate	1278	-	0.32
α-Terpinyl acetate	1302	0.32	-
Piperitenone	1329	0.37	1.32
β-Elemene	1352	0.33	0.12
β-Caryophyllene	1425	-	0.47
α-Humulene	1466	0.67	-
Germacrene D	1482	0.34	0.46
Elemol	1521	0.39	0.67
Spathulenol	1548	-	0.16
α-Cadinole	1567	-	0.23
Total		99.75	98.28

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C8-C17) to HP-5MS column.

Mill. from Kerman province, Iran were *trans*-anethole and Fenchyl acetate. Extensive research has been conducted on the chemical composition of volatile oils of fennel major constituents of sweet fennel (var. *dulce*) and bitter fennel (var. *vulgare*) to include anethole, estragole, and fenchone plus an additional 18 compounds extracted in the monoterpene fraction of the fruit. The minor constituents accounted for 1 to 5% of the total oil of the volatile oil and included: α-pinene, camphene, β-pinene, α-phellandrene, myrcene, limonene, β-phellandrene, γ-terpinene, *cis*-ocimene, terpinolene, and *P*-cymene.

Significant differences in oil composition between var. *dulce* and *vulgare* were noted (Karlsen et al., 1969). In a comparison 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 (Bernáth et al., 1996). In a research seed oil composition of *F. vulgare* was reported to be influenced by growing region (Arslan et al., 1989).

In a study the chemical composition of stems, leaves and seeds of *F. vulgare* Miller from two Spanish locations (Santander and Aranjuez) were investigated. 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. The oil from the leaves collected in Aranjuez (April, June) was found to contain methyl chavicol (12.3 to 12.1%), α-phellandrene (9.4 to 27.2%), limonene (25.3 to 18%) and fenchone (19.4 to 18.3%) as major constituents. The oil from the stems gathered in Aranjuez (September) was characterized by a high content of (E)-anethole (17.4 to 3 to 3%), α-pinene (9.7 to 14.4%), α-phellandrene (24.3 to 31.4%), p-cymene (11.5 to 5 to 2%), limonene (11.6 to 15%) and fenchone (9 to 1 to 17 to 5%) (García-Jiménez et al., 2000).

In a research the main identified components of the flower and unripe and ripe fruit oils of *F. vulgare* ssp. *piperitum* were estragole (53.08, 56.11, and 61.08%), fenchone (13.53, 19.18, and 23.46%), and *α*-phellandrene (5.77, 3.30, and 0.72%) (Özcan et al., 2007). It is reported that the major oil constituent of sweet fennel fruit was *trans*-anethole, estragole, fenchone, *P*-anisaldehyde and limonene (Katsiotis, 1988). Volatile oil extracts of fennel seeds were *trans*-Anethole (anise, licorice), estragole (anise, licorice, sweet), fenchone (mint, camphor, warm), and 1-octen-3-ol (mushroom) were the most intense odor compounds detected in fennel extracts (Daz-Maroto et al., 2005). 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%) (Damjanović et al., 2005).

In a study, main compound of the essential oil of fennel leaves, flowers, fruits and stems were (E)-anethole varied from 50.1% in fruits to 73.6% in stems. Limonene (1.5 to 20.7%), fenchone (3.6 to 15.9%), α-pinene (1.9 to 19.5%) and α-phellandrene (2.4 to 8.8%) were the other quantitatively important constituents (Venskutonis et al., 1996). It is reported that *trans*-Anethole was the main volatile compound of fennel (*F. vulgare* Mill.) (Díaz-Maroto et al., 2006). In other study main composition of annual sweet fennel (*F. vulgare* var. *dulce* Mill.), were *Trans*-anethole, fenchone, estragole and limonene represented 79.2% of the herb and 93.1% of the fruit (Embong et al., 1977).

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