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Comparison of Seed Essential Oil Content in Five Populations of Fennel (Foeniculum vulgare Mill.)

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Abstract

The purpose of this research was to investigate the type and percentage of active ingredient (active matter, effective material) in five seed populations of fennel (Foeniculum vulgare Mill), which were collected from different regions. Seed essential oil extraction was performed through hydrodistillation using a Clevenger apparatus. The seed extract compounds were determined using GC-Mass. The result indicated a genetic variation among the studied populations in terms of essential amount and component. Limonene, alpha-Phenchone, trans-anethole, and estragole were major compounds of the studied populations. Among these compounds, limonene was known as an aromatic agent with antiseptic and anti-inflammatory properties, trans-anethole was identified as an aromatic agent with antiseptic and anti-cancer properties as a useful ingredient, and estragole was recognized as an aromatic agent, but carcinogenic compound. Tabriz population had the highest amount of limonene and the German population had the highest amount of alpha-Phenchone as much as 13.042% and 12.64%, respectively. The highest amount of trans-anethole was related to Izmir population and the highest amount of estragole belonged to Neyshabur population as much as 80.677% and 63.728%, respectively. The lowest amount of active ingredient obtained from the seed extracts of the studied populations was related to Pologne, Furanone, Pyridine, Methyl, and Pentene.

Keywords: Fennel, Essential oil, GC-Mass, Trans-anethole, Limonene

Medicinal plants are one of the important sources of medicine production, which have been used by humans for many years. Today, they have not only lost their value in medicine production but also have become

increasingly important. Secondary metabolites are complex organic chemicals including acids, lactones, flavonoids, terpenoids, and anthocyanins that plants produce throughout their lives (Kumar and Gupta, 2008). The fast and mass production of some of the secondary metabolites is not possible on a large scale by chemical methods due to their complex structure as well as technical difficulties. Therefore, the only source of production of these metabolites is the plant cell (Ebrahimpour and Eidizadeh, 2009).

Fennel (Foeniculum vulgare Mill.) is one of the most important and widely used medicinal plants of the Apiaceae family. Fennel has become naturalized in southern Europe and the Mediterranean region and grows in France, Spain, Portugal, and North Africa as a wild plant (Kumar et al., 2002). Fennel is a perennial and diploid plant with a chromosome number of 2n=2x=22 (Mozaffarian, 1996), which is mainly cropped to use its essential oil in in the pharmaceutical, food, cosmetic and hygiene industries (Darzi and Haj Seyed Hadi, 2001). Fennel has been documented as a medicinal plant, which is used in traditional medicine as well as an effective medicinal plant in leading pharmacopoeias worldwide (Safai Khorram et al., 2008; Shah et al., 1992). Studies on the properties of fennel have demonstrated that the resulted essential oil has strong antimicrobial and antioxidant activity (Embong et al., 1977; De Maniro et al., 2007). Fennel essential oil contains phenolic ethers, which is the main cause of its medicinal properties. The fruit of this plant is used as an antispasmodic, energizer, sedative, and breast milk enhancer. Fennel fruit has a kind of Estragoleic effect, which can lead to weight gain and obesity (Lawless, 1992).

The most commonly used part of it is the dried ripe fruit and rarely its dried root, excessive consumption causes poisoning and immediate vomiting or allergy (Tavakoli Saberi, 2000; Salehi Surmaqi, 2006). The active ingredient of fennel is its essential oil, which contains numerous Terpenoids. Singh et al. (2006) identified 35 compounds in fennel essential oil that made up 96.4% of the essential oils compounds. The whole plant has essential oil, but its fruit has the highest amount of essential oil. The essential oil of the plant is in the channel-type structure created by the glandular cells and scattered throughout the plant. Most of these channels are in the seed coat (Charles et al., 1993; Moura et al., 2005). Fennel volatile oil or essential oil is a yellow liquid that is obtained by seed or fruit distillation through vapor. Fennel fruit contains 2 to 6% essential oil. Fennel fruit contains about 10% oil, 4% Palmitic acid, 22% oleic acid, 14% linoleic acid, 60% Petroselinic acid as well as minerals such as Hosphorus, potassium, magnesium and manganese, sugars, mucilage, gum, oils, vitamins A, B, C, lime and carbohydrate (Amin, 1991; Yazdani et al., 2004). The most important composition of the essential oil of fennel is Anethole, which plays a decisive role in its essential oil quality (Gross et al., 2002). Limonene and Methyl-Chavicol (estragole) can be mentioned as the other important compounds of this plant (Darzi et al., 2008). Fennel essential oil consists of Alpha-Pinene, Camphene, Alpha-Phellandrene, Trans-Anethole, Phenchone, Estragole, Anisaldehyde and finally small amounts of some alkaline compounds that make up the dominant compound in some varieties. Essential oil compounds are usually varied in different varieties of fennel (Jaymand and Rezaei, 2001; Stahl, 1975).

The purpose of this research was to compare the amount and type of active ingredients in the seeds of five studied populations.

Research Method

Plant materials were the seeds of five Fennel populations including the German

fennel (1182), Izmir Turkey, Gerine (Gharineh) Neyshabur, Khorshidabad, Meshkinshahr, and Tabriz. The essential oil extraction was performed in the laboratory of the Faculty of Agriculture of Tabriz University and a factorial experiment was done in a completely randomized design with five iterations. The evaluated traits included percentage and duration of seed germination as well as identification and percentage of the compounds of essential oil extracted from the seeds and their comparison with each other.

Seed Essential Oil Extraction

Seed essential oil extraction was performed through hydrodistillation using a Clevenger apparatus. Initially, 100g of the fennel seeds were crushed by electric grinding and then passed through sieve No. 16 to uniformize the particle size. The prepared powder was thoroughly mixed in the two-liter round bottom flask with distilled water and attached to the essential oil extractor. This process lasted for 180 minutes. The essential oil could be measured after five minutes in the scaled part of the tube. The contents of the interface tube were transferred to a 10ml scaled cylinder and the essential oil volume was determined in milliliters. Given the lightness of the essential oil and its placement on water, it was then separated from the surface of the water by Pasteur pipette and covered n small glass jars with aluminum foil. The essential oil was injected into the GC-Mass device to determine the compounds.

The applied GC-Mass device N6890Aglient was HB-5MS silica column with 30m length, 0.25mm thickness, and 0.25µm film thickness. 95% helium gas is used as GC carrier gas. The essential oil was injected using a 2µm syringe.

Statistical Analysis

Prior to analyzing the variance of the data, the assumptions were examined and confirmed. Then, the data were analyzed in the form of a completely randomized design with five iterations. Excel, C-MSTAT, and SPSS software were used for data analysis and plotting.

Findings

According to the analysis results of essential oil from five populations of fennel seed studied by GC-Mass device, Limonene, alpha-Phenchone, trans-anethole, and estragole were the major seed components of the studied populations (Tables 1 and 2).

Table 1. Active ingredients in five populations of Fennel

Active material of seeds															Population
Camphor	Furanone	Pulegone	Copaene	Alpha-phencho	Beta-	Gamma-	Delta-	Limonene	Beta-	Sabinene	Camphe	Propioni	Alpha-	Alpha-	
0.111	-	-	0.068	-	0.12	0.151	-	7.493	0.495	0.501	0.087	-	1.405	0.185	Neyshabur
0.044	-	-	-	1.989	0.08	0.131	-	4.57	0.252	0.309	0.034	-	0.746	0.168	Ezmir
0.170	-	-	-	0.032	0.13	0.480	0.064	13.042	0.460	0.427	0.086	-	1.616	0.176	Tabriz
0.330	0.014	0.01	0.093	10.655	-	0.109	-	5.046	0.727	0.261	0.240	0.952	-	0.2	Khorshidabad
0.590	-	-	-	12.62	0.01	1.030	0.054	11.093	0.230	0.300	0.170	0.004	1.20	0.41	German

Table 2. In the following of Table 1

Active material of seeds															Population
Unknow	Pay-	Pentene	Menthol	Linalool	Toluene	Estragole	Sis-	Thyocyn	Delta-	Germacr	Methoxy	Alpha-	Phenchil	Trans- anethole	
4.081	0.040	-	-	-	2.055	63.728	1.849	0.018	0.008	0.093	0.089	0.008	0.305	15.23	Neyshabur
1.325	-	-	-	-	0.024	6.343	0.901	0.007	-	0.110	-	-	0.046	80.677	Ezmir
0.128	-	-	-	-	0.062	56.732	0.773	5.416	0.011	0.091	0.263	0.023	0.331	19.487	Tabriz
0.197	0.030	-	-	-	-	5.396	1.021	0.011	0.012	0.163	0.165	-	0.056	74.312	Khorshidabad
0.629	-	0.003	0.047	0.65	0.277	5.540	-	-	0.010	0.082	0.061	0.065	0.084	64.22	German

Discussion and Conclusion

According to the results of the essential oils of the five populations studied, Tabriz population had the highest amount of Limonene as much as 13.042% and German population had the highest amount of alpha-Phenchone as much as 12.62%. The highest amount of trans-anethole was related to Izmir population as much as 80.677$ and the highest amount of estragole belonged to Neyshabur population as much as 63.728%. Pologne, Furanone, Pyridine, Methyl, and Pentene had the lowest amount among the active ingredients obtained from the seed extract of the studied populations. The lowest amount of Furanone was observed in Khorshidabad population as much as 0.014. The German population showed the lowest amount of Pologne, Alpha-terpineol, Pyridine, and Pentene as much as 0.02, 0.024, 0.004, and 0.003, respectively.

Omid Beigi (1997) introduced fennel seed essential oil consisting of more than 30 types of Terpène compounds, the most important of which were Trans-Anethole, Phenchone, Limonene, Methyl-Chavicol, Estragole. According to Julien and Manazonus (1996), fennel fruit also contains a little sugar, mucilage, and essential oil as much as 4-6% and the amount of essential oil and its compounds vary according to the plant's location. Shams Ardakani et al. (2004) identified 37 compounds in the essential oil of fennel seed according to the study of mass spectra obtained from GC-Mass, which totally composed 91.37% to 96.93% of the essential oil. The main compound of fennel seed essential oil was Trans-Anethole (47.89 to 50.18%), Estragole (7.63 to 8.86%), Phenchone, (7.63 to 8.86%), and Limonene (6.72 to 8.86%). Jamshidi et al. (2004) reported about 91.37 to 97.57% of volatile oil by investigating the effect of essential oil extraction time on volatile oil compounds of fennel seed via GC-Mass. Among the identified oils, Anethole (47.89 to 62.73%), Estragole (9.41 to 16.52%), and Phenchone (7.63 to 8.86%) composed the highest percentage of the materials. Kirci et al. (2010) examined the compound and percentage of essential oil components of four wild fennel populations of Turkey (Ceyhan, Yumurtalyk, Antakya, and Belen). The amount of essential oil obtained from the seeds of these populations was about 5.1 to 6.4%. In this research, the essential oil analysis was studied using GC-Mass in which Pinene, Myrcene, Limonene, Phenchone, Estragole, and Trans-Anethole were the six

main compounds of each population with different percentages. Anvar et al. (2009) investigated the antioxidant and antimicrobial activity of essential oil and extraction of fennel seed using GC-Mass device and found 23 compounds in Pakistani fennel seed oil, of which Trans-Anethole (69.87%), Phenchone (10.23%), Estragole (5.45%), and Limonene (5.10%) were the main compounds. Shams Ardakani et al. (2005) stated that the amount of seed extract varies from 1.75 to 4% and its main components include Caren, Limonene, and Alpha-Thujene. Abou El-Nasr et al. (2013) evaluated the genetic variation of the three varieties of Holand, Balady, and Indian based on appearance traits and essential oil compounds, and ISSR marker. Most seeds of Holand, Balady and Indian cultivars had the highest genetic value, respectively. Seven different compounds were identified in the essential oils of cultivars by GC-Mass analysis. Methyl-Chavicol and Trans-Anethole were identified as the main compound of oil in these three fennel cultivars. The highest amount of Chavicol was produced by Balady and then, by Indian and Holand cultivars, respectively. Radulovic and Blagojevic (2010) identified 89 compounds with GC-Mass by analyzing the secondary volatile metabolites of fennel as well as essential oil decomposition of root and schizocarp and a quarter of fennel compounds were identified as volatile compounds for the first time. The samples were mostly composed of phenylpropanoid13 (69.5-85.5%) and monoterpenoid14 (11.7-26.9%), the dominant metabolites were schizocarp, Phenchone (13.3-18.8%) and Anethole (0.69-1.66%), and the main compounds of fennel root were terpinolene15 (6.2-6.5%), and diapiol16 (71.4-77.5%). Ozcan et al. (2006) compared the compounds of the essential oil of the fennel flower, unripe and ripe fruit of bitter fennel (F. vulgare ssp. Piperitum) by GC-Mass. They reported Estragole (53.80, 56.11, and 61.08%), Phenchone (13.53, 19.18, and 23.46%), and Alpha-Phellandrene (5.77, 3.30, and 0.72) as the main compounds of the essential oil of flower, unripe and ripe fruit.

Conclusion

- The studied populations had significant differences in percentage and duration of seed germination.
- Trans-Anethole is one of the important compounds of fennel seed, which was observed in the seed of all studied populations.

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