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Original Article

The Effect of Habitat Altitude on Quantity and Constituents of Root Sap Essential Oil in *Ferula foetida* L.

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ABSTRACT

Ferula foetida (Apiaceae) is a medicinal plant whose oleo-gum-resin is useful in medicine. In order to investigate the effect of habitat altitude on quantity and constituents of root essential oil of *F. foetida*, we selected six samples of 7-year-old roots of *F. foetida*, which were collected from 6 ecotypes located in Razavi Khorasan province (Dergaz, Kalat, Bajestan, Sabzevar, Neishabur, and Kashmar), Iran in April 2013. we extracted Essential oil of 6 root samples using the hydro-distillation method and analyzed by capillary gas chromatography and mass spectrometric detections. Result showed that average weight of Essential oil of *F. foetida* root for Bajestan, Kashmar, Sabzevar, Nishabour, Dargaz, and Kalat samples obtained 0.27%, 0.13%, 0.30%, 0.08%, 0.20%, and 0.15%, respectively. Also, there was a negative correlation between altitude of the ecotypes and the weight of the Essential oil of root sap. GC–MS for *F. foetida* root Essential oil showed that there are 24 compounds in the Bajestan sample and 27 compounds in Kashmar and Nishabour samples. Z-propenyl-sec-butyl disulfide, e-Propenyl-sec-butyl disulfide, and α-humulene were common in all samples. Also, 3 to 5 compounds found in the root Essential oil of some *F. foetida* ecotypes, which were not in all samples. Sesquiterpenes and monoterpenes had the most abundant and esters and thioethers had the least numerous in *F. foetida* sap Essential oil. Other results showed that the amount of Essential oil in *F. foetida* root decreases with the increase of the altitude of its habitat, but this factor did not show a clear trend on the number of chemical compounds of *F. foetida* root Essential oil.

INTRODUCTION

Ferula from Apiaceae has about 170 species in the world [1]. About 30 species of *Ferula* have been reported in Iran, 15 of which are native to Iran [2]. *Ferula* as one of the most promising genera of Apiaceae with many species is sources of aromatic oleo-gum-resins that have been valued since antiquity as remedies, condiments and incense [3]. One of the *Ferula* species whose sap has medicinal and industrial properties is *F. foetida* that grows in some rangelands of Iran [1,2]. Most *Ferula* species have sap which has phytochemical and biological properties [4]. Phytochemical analyses of *Ferula* sap have confirmed the presence of sesquiterpene coumarins [5,6], sesquiterpenes [7,8], sulfides and volatile oils [9,10]. In scientific references, the sap of the *F. foetida* is called oleo-gum resin which consists of gum (glucose, galactose, L-arabinose, rhamnose and glucuronic acid, etc.), resins (ferulic acid esters, free ferulic acid, coumarin derivatives), and volatile oils (sulphur-containing compounds and various monoterpenes) [11]. *F. foetida* also has a local name and it is called Anghuzeh (in Farsi) [12]. In terms of botanical characteristics, *F. foetida* is a perennial plant with compound leave. It is a hemicryptophyte, perennial, and monocarpic plant with yellow flowers, schizocarp fruit, bulky root, and a 1-1.5 m tall stem. There is oleo gum resin obtained from the stem and root of the plant. *F. foetida* gum, which seeps naturally from the stem, is used in the pharmaceutical and industrial industries [13, 14]. The exuded substance (oleo-gum resin) by this plant has been traditionally used for the treatment of a vast range of diseases (urinary, gastrointestinal and respiratory infections, and epilepsy), as well as an aphrodisiac, an emmenagogue, and also to treat snake and insects' bites, with the best-documented folk use being the management of intestinal worm infections [15, 16]. There are several reports on the pharmacological activities of *F. foetida* such as antiviral (HSV, HRV, H1N1, HIV), antispasmodic, hypotensive, and antidiabetic [17]. One of the characteristics of its essential oil (Essential oil) is the existence of volatile sulfide constituents, a type of non-ubiquitous compounds with significant pharmacological effects [18,19]. One of the environmental factors that affect the growth of plants is altitude. Since altitude affects climatic and edaphic factors, then it can impress canopy cover size, height, density, as well as the quantity and quality of plant by-products [20]. *F. foetida* habitats are scattered in most of the semi-arid zone in Iran. Reviews showed that no study has been conducted on the altitude distribution of *F. foetida* habitats and its effect on the quantity and quality of its oleoresin essential oil. The purpose of this study is to investigate and compare the quality and quantity of *F. foetida* root sap essential oil in six habitats located in Razavi Khorasan province, Iran.

MATERIALS AND METHODS

Plant Material

In April 2021, six samples of 7-year-old roots of *F. foetida* were collected from 6 habitats (ecotypes) located in different altitudes of Razavi Khorasan province, Iran (Fig. 1) [21]. In the next stage, the samples were transferred to the medicinal plants laboratory of Khorasan Razavi Agricultural Research Center, Mahshad, Iran to extract their Essential oil. The physiographic characteristics of *F. foetida* habitats are given in Table 1.

Isolation of the Essential Oils

For isolation of the Essential oil from root samples, 500 g of *F. foetida* fresh root was selected from each sample and milled using a mill (Model PX-MFC90D) according to following analysis [22]. The Essential oil extraction operation were performed by a Clevenger-type apparatus based on hydro-distillation (European pharmacopeia, 1998). The duration of the distillation was done for 1.5 hours. This experiment was carried out with three replications and the average weight and volume of Essential oil were calculated for each sample (Table 1). Then the Essential oil were kept in a dark glass vial at 4 ˚C for GC–MS examinations.

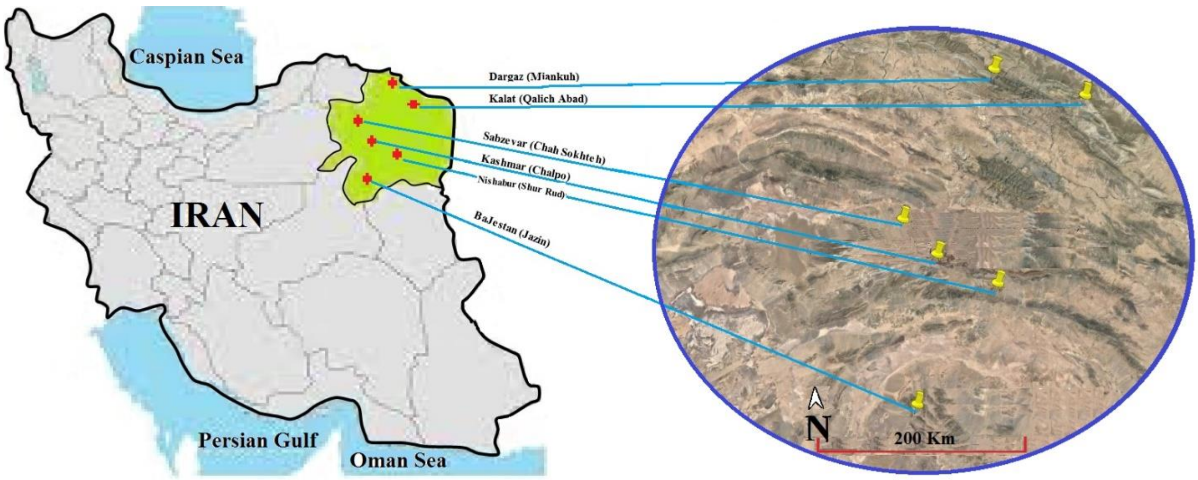

Fig. 1 *F. foetida* root collection sites

Table 1 The physiographic characteristics of *F. foetida* root collection sites

Name of area	Altitude (meter)	Zone	Latitude	Longitude
Dargaz (Miankuh)	1255	40 S	682300.00 m E	4116107.00 m N
Kalat (Qalich Abad)	1307	41 S	234338.00 m E	4088468.00 m N
Bajestan (Jazin)	1403	40 S	597035.00 m E	3801012.00 m N
Sabzevar (Chah Sokhteh)	1423	40 S	590345.00 m E	3965473.00 m N
Nishabour (Shur Rud)	2056	40 S	644990.00 m E	3946476.00 m N
Kashmar (Chalpo)	2118	40 S	640151.00 m E	3944152.00 m N

Gas Chromatography

GC analyses were performed using gas chromatography, Agilent 7890A GC system for identification of the compositions of two Essential oil samples. The Essential oil were analyzed by GC-MS, Thermo-UFT model, in the central laboratory of Research Institute of Forest and Rangelands, Tehran, Iran. Profile column machine brand HP-5, capillary column, manufactured by Agilent ® J&W™ HP-5 Scientific with Length of 30 m, an inner diameter of 0.25 mm (ID), film thickness: 0.25 µm. The inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Column temperature program: initial temperature 60 °C to start the final temperature of 220 °C. The initial 3 °C per minute to be added and then injected into the chamber to a temperature of 260 °C. Rate: 20 °C/min, hold time: 10 min. Inlet temperature: 260 °C, Split ratio: 40:1. Injection volume: 0.1 µL. Carrier gas: Nitrogen, Flow rate: 0.7 ml/min. Detector (FID) temperature: 260 °C. The carrier gas inlet pressure to the column: helium with a purity of 99/99% of the inlet pressure to the column equal to 5/1 kilogram per square centimeter is set [23].

Gas Chromatography-Mass Spectrometry

GC–MS analysis is performed on an Agilent 7890A/5975C GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature is programmed as follows: the initial temperature of 60 °C is immediately increased to 220 °C at a rate of 3 °C/min; subsequently the temperature is increased to 260 °C at 20 °C/min and held at this temperature for 3 minutes. The injector and transfer line temperature are 260 and 280 °C, respectively; carrier gas is helium with a linear velocity of 30.6 cm/s; split ratio 1:100, ionization energy 70 eV, scan time 1s, mass range 30–340 a.m.u. The identity of the volatile oil composition was established from their GC retention indices, relative to C7- C25 n-alkanes standards mixture, and by comparison of their mass spectra and retention indices with those reported in the literature [24, 25] and by computer matching with the Wiley 5 and NIST mass spectra library, whenever possible, by co-injection with standards available in the laboratories. Finally, the identified compounds obtained from the analysis of essential oil samples were recorded based on the Kovats index (Table 3).

RESULTS

The results showed that *F. foetida* sap Essential oil has a bright red color and a sulfurous pungent odor. The highest and the lowest average of weight and volume of the sap Essential oil extracted from *F. foetida* root samples be were observed from Sabzevar and Dargaz ecotypes, respectively (Table 2).

The results also suggested that there is a negative correlation (r = -73%) between altitude of the habitats of *F. foetida* and the weight of the essential oil of its root sap. So that the amount of essential oil of *F. foetida* root decreases with the increase of the altitude of its habitat (Fig. 2).

GC–MS analysis for *F. foetida* root sap Essential oil showed that there are at least 24 chemical compounds in the Bajestan (Jazin) sample and a maximum of 27 chemical compounds in the Kashmar (Chalpo) and Nishabour (Shur Rud) samples (Fig. 3, 4, and 6 & Table 3). Generally, 24 to 27 chemical components were characterized in the samples collected from 6 habitats of *F. foetida* (Table 3). The identified compositions belong to five major chemical groups including terpenes, esters, ethers, organic sulfides, and an organic compound were listed in order of their elution on the HP-5MS column with their percentage values (Table 3).

Results suggested that z-propenyl-sec-butyl disulfide, e-Propenyl-sec-butyl disulfide, and α-humulene were common in all samples (Table 3). As it is observed, z-propenyl-sec-butyl disulfide and e-Propenyl-sec-butyl disulfide in the composition of *F. foetida* root sap essential oil were the highest amount of all for the samples related to Bajestan, Kashmar, Sabzevar and Nishabour, but they were not much in the sample of Kalat (Fig. 8).

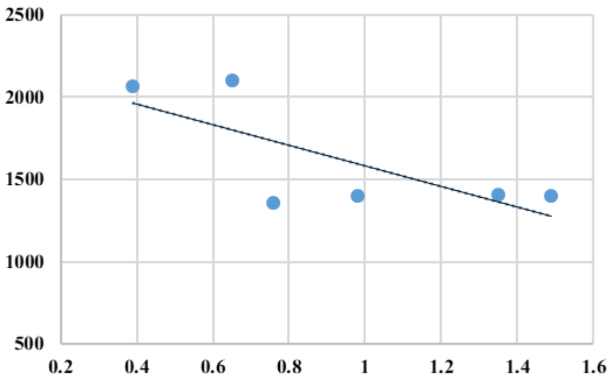

Fig. 2 The relationship between the altitude of the habitat of *F. foetida* and the weight of the essential oil of its root sap

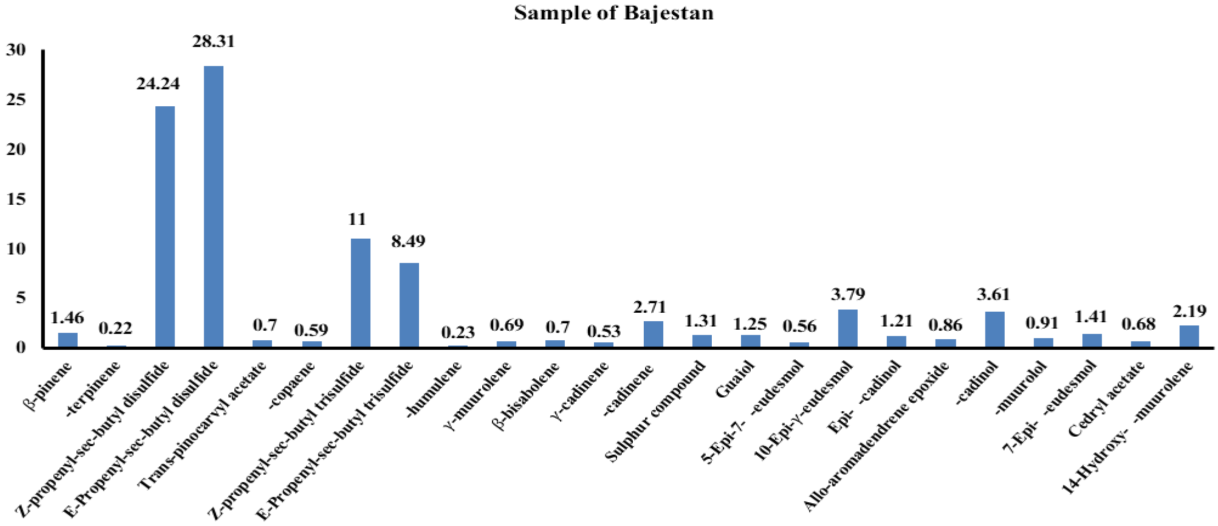

Fig. 3 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Bajestan (Jazin)

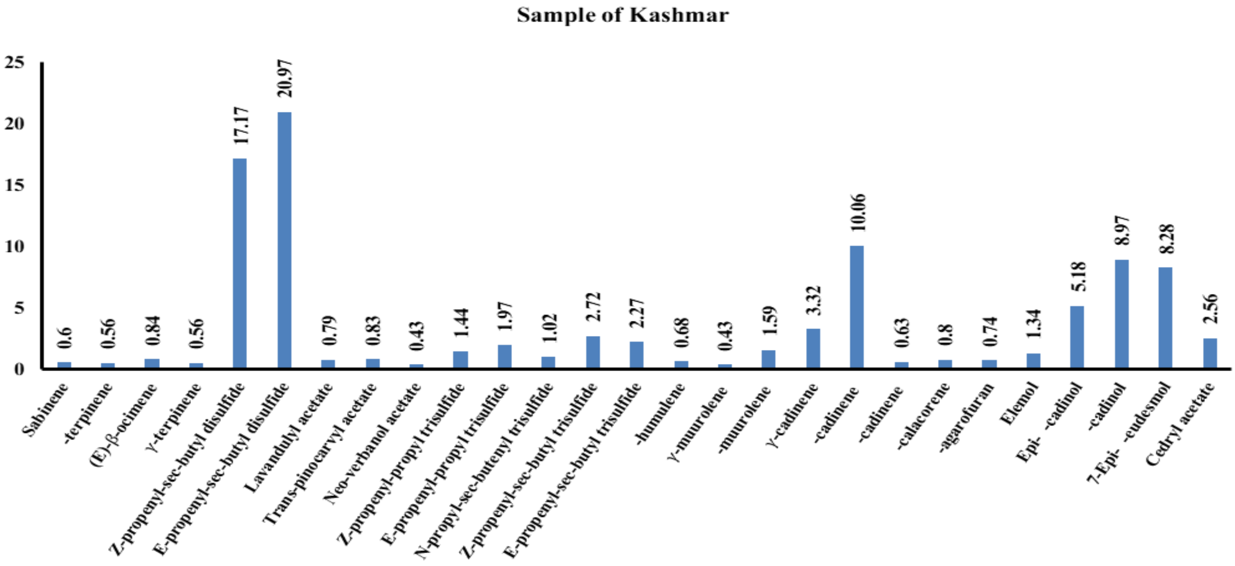

Fig. 4 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Kashmar (Chalpo)

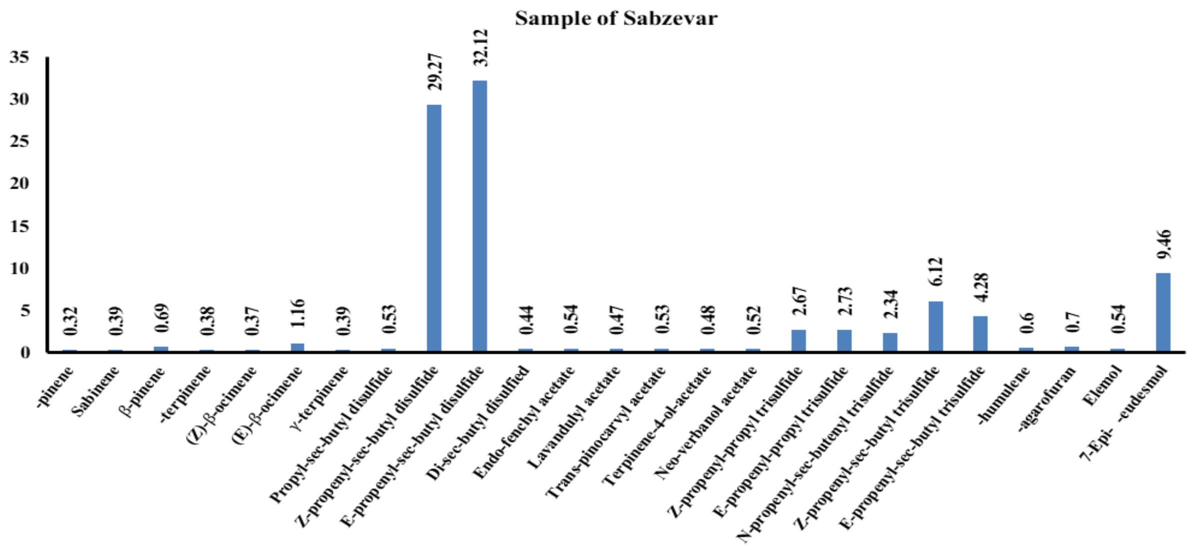

Fig. 5 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Sabzevar (Chah Sokhteh)

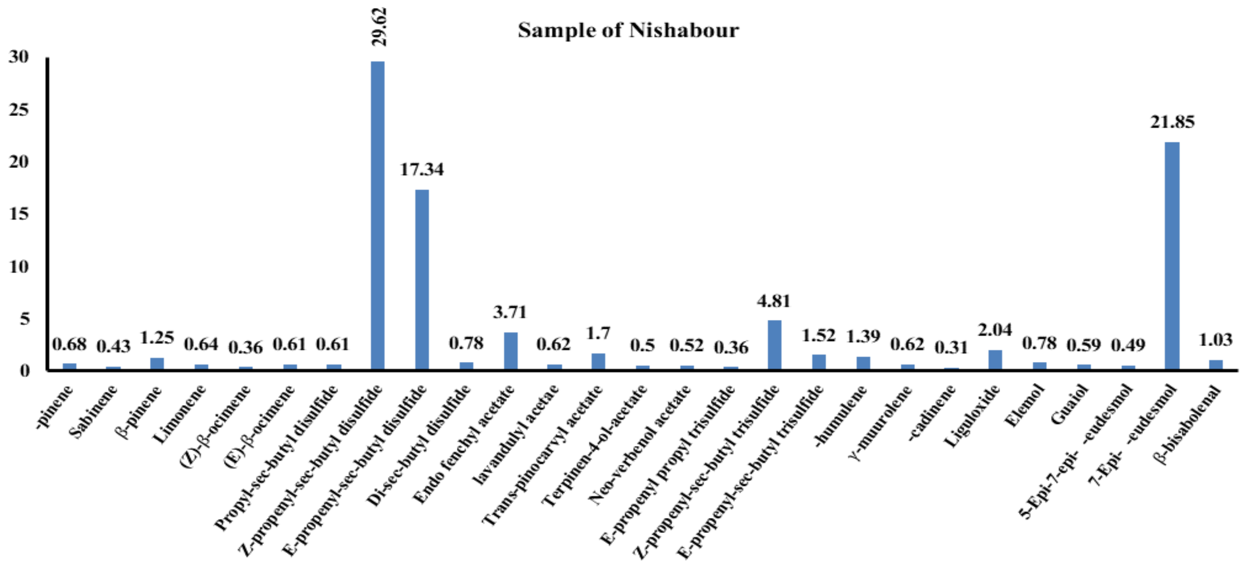

Fig. 6 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Nishabour (Shur Rud)

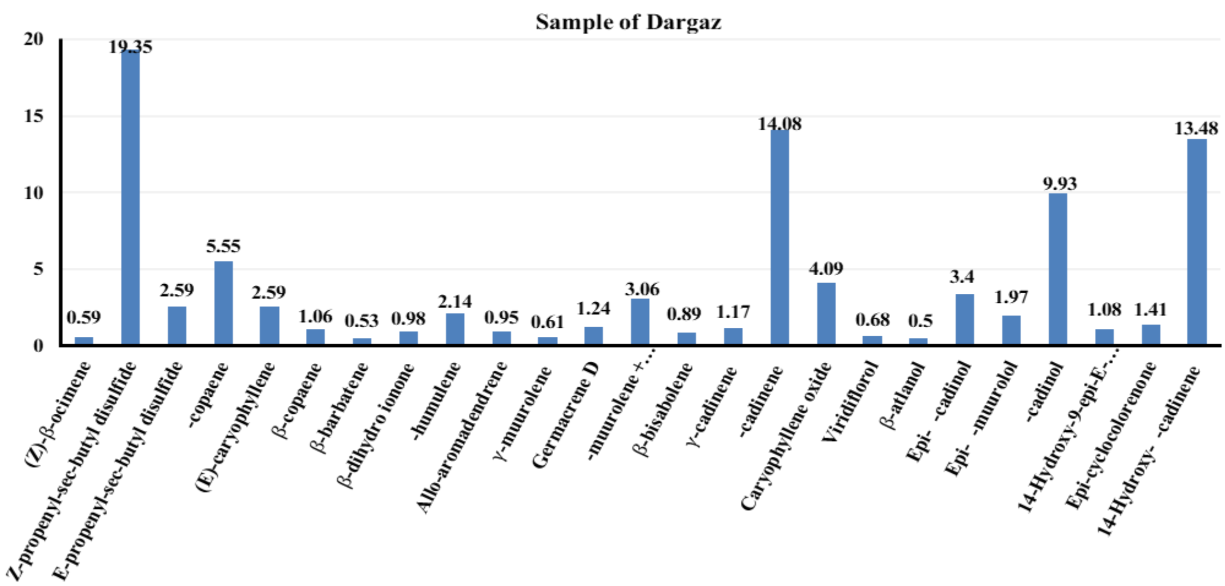

Fig. 7 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Dargaz (Miankuh)

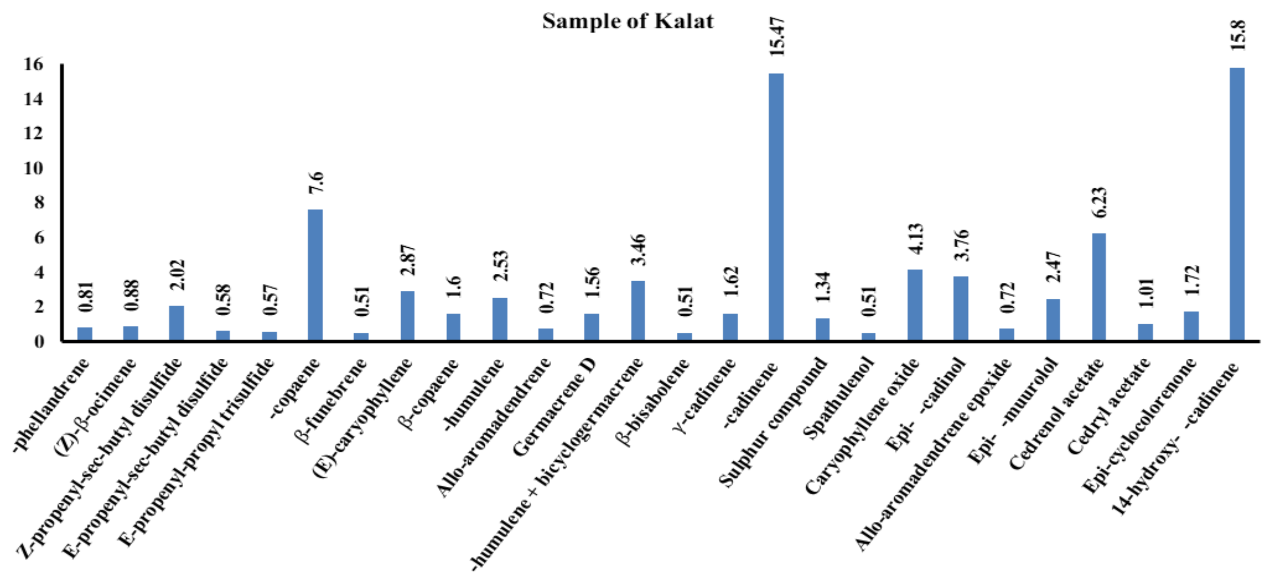

Fig. 8 Identified constituents of root sap Essential oil in the *F. foetida* for sample of Kalat (Qalich Abad)

This comes as they were 19.35% and 9.59%, respectively in the sample of Dargaz (Fig. 7). Based on the results obtained from this research, the highest amount of e-propenyl-sec-butyl disulfide (32.12%) was observed in Sabzevar sample (Fig. 5) and the lowest amount was observed in Kalat sample (0.58%) (Fig.8).

Also, we found some chemical compounds that were unique to the essential oil of one habitat such as (5-Epi-7-α-eudesmol, 10-Epi-γ-eudesmol, α-muurolol, Cedryl acetate, and 14-Hydroxy-α-muurolene) in Bajestan sample, (α-muurolene, α-cadinene, and α-calacorene) in Kashmar sample, (Limonene, Liguloxide, and 5-Epi-7-epi-α-eudesmol in Nishabour sample, (β-barbatene, β-dihydro ionone, Viridiflorol, and 14-Hydroxy-9-epi-E-Caryophyllene) in Dargaz sample, and (α-phellandrene, β-funebrene, Spathulenol, and Cedrenol acetate) in Kalat sample (Table 3 and Fig. 9).

In this research, sesquiterpenes and monoterpenes were the most and esters and thioethers were the least compounds of *F. foetida* essential oil (Table 3). Also, GC-MS analysis of *F. foetida* essential oil revealed some unknown sulfated compounds whose RIs were not found in the Quats RI database (eg: 1541, 1542, 1549 and 1550). Therefore, these substances are marked as ND (not diagnosed) in Table 3.

Table 2 Weight and volume of sap Essential oil per 500 g of fresh *F. foetida* root for samples collected from 6 habitats located in different altitudes of Razavi Khorasan province, Iran

Sample location	Essential oil weight (gr)	Volume of Essential oil (ml)	[Essential oil weight (gr)/ Root dry weight (gr)] * 100 or (v/w)
Bajestan (Jazin)	1.35	1.43	0.27
Kashmar (Chalpo)	0.65	0.69	0.13
Sabzevar (Chah Sokhteh)	1.49	1.55	0.30
Nishabour (Shur Rud)	0.39	0.43	0.08
Dargaz (Miankuh)	0.98	1.04	0.20
Kalat (Qalich Abad)	0.76	0.71	0.15

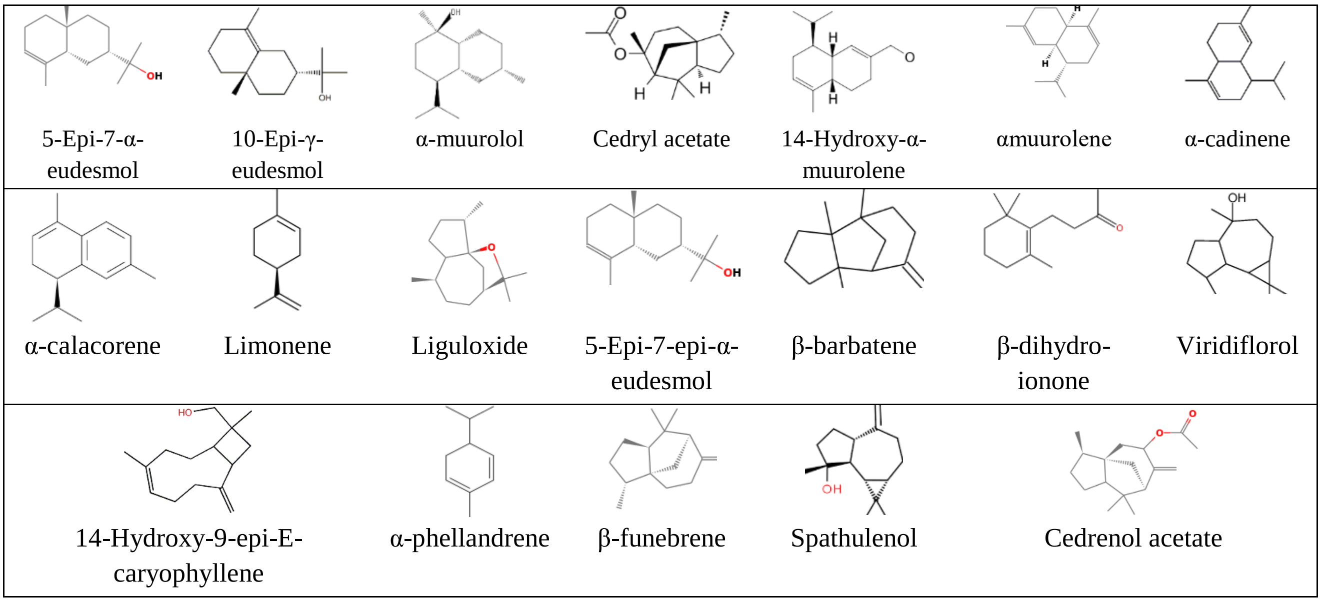

Fig. 9 The molecular structure of some chemical compounds that were unique to the essential oil of one habitat (Table 3) [26]

Table 3 Comparison of percentages and compositions of root sap Essential oil in *F. foetida* for 6 habitats in Khorasan Razavi province, Iran

Compounds name	Chemical compound	(RI*)	The percentage and RI of essential oil compounds in the studied habitats					
			Bajestan (Jazin)	Kashmar (Chalpo)	Sabzevar (Chah Sokhteh)	Nishabour (Shur Rud)	Dargaz (Miankuh)	Kalat (Qalich Abad)
			Area% (GC)	Area% (GC)	Area% (GC)	Area% (GC)	Area% (GC)	Area% (GC)
α-pinene		932-938	-	-	0.3	0.7	-	-
Sabinene		969-971-973	-	0.6	0.4	0.4	-	-
β-pinene		974-982	1.5	-	0.7	1.3	-	-
α- phellandrene		1002-1009	-	-	-	-	-	0.8
α-terpinene		1014-1015-1017	0.2	0.6	0.4	-	-	-
limonene		1024-1034	-	-	-	0.6	-	-
(Z)- β-ocimene		1032-1036-1037	-	-	0.4	0.4	0.6	0.9
(E)- β-ocimene		1044-1048-1049	-	0.8	1.2	0.6	-	-
γ-terpinene		1054-1061-1062	-	0.6	0.4		-	-
Propyl-sec-butyl disulfide		1155	-	-	0.5	0.6	-	-
Z-propenyl-sec-butyl Disulfide		1158-1160	24.2	17.2	29.3	29.6	19.4	2.0
E-propenyl-sec-butyl disulfide		1162-1165	28.3	21.0	32.1	17.3	2.6	0.6
di-sec-butyl disulfide		1215-1216	-	-	0.4	0.8	-	-
Endo- fenchyl acetate		1220-1221	-	-	0.5	3.7	-	-
Lavandulyl acetate		1281-1282-1289	-	0.8	0.5	0.6	-	-
Trans-pinocarvyl acetate		1296-1297-1299	0.7	0.8	0.5	1.7	-	-
Terpinene-4-ol acetate		1298-1299-1340	-	-	0.5	0.5	-	-
Neo-verbanol acetate		1315-1319	-	0.4	0.5	0.5	-	-
Z-propenyl -propyl trisulfid		1343-1344	-	1.4	2.7		-	-
E-propenyl -propyl trisulfid		1346	-	2.0	2.7	0.4	-	0.6
α- copaene		1363-1364-1376	0.6	-	-	-	5.6	7.6
n-propyl- sec-butyl Trisulfide		1377	-	1.0	2.3	-		-
β- funnebrene		1410-1419	-	-	-	-		0.5
(E)- caryophyllene		1417-1422-1423	-	-	-	-	2.6	2.8
Z-propenyl-sec-butyl Trisulfide		1432-1433	11.0	2.7	6.1	4.8	1.1	1.6
E-propenyl-sec-butyl trisulfide		1434-1436	8.5	2.3	4.3	1.5	-	-
β- barbatene		1441-1457	-	-	-	-	0.5	-
β- dihydroionone		1433-1454	-	-	-	-	1.0	-

Table 3 (Continue)

α- humulene	1454-1460	0.2	0.7	0.6	1.4	2.1	2.5
Allo-aromadendrene	1461-1463					1.0	0.7
γ- muurolene	1472-1477	0.7	0.4	-	0.6	0.6	0.8
Germacrene D	1480-1482	-		-	-	1.2	1.6
α- muurolene	1497-1499	-	1.6	-	-	3.1	3.5
β- bisabolene	1503-1504-1508	0.7		-	-	**0.9**	**0.5**
γ- cadinene	1511-1513	0.5	3.3	-	-	1.2	0.9
δ- cadinene	1515-1517-1524	2.7	10.1	-	0.3	14.1	15.5
α- cadinene	1520-1538	-	0.6	-	-	-	-
α- calacorene	1533-1542	-	0.8	-	-	-	-
Sulfur compound = ND	1541-1542	0.9	-	-	-	-	6.0
Sulfur compound = ND	1549-1550	0.5	-	-	-	-	0.7
α- agarofuran	1545-1556	-	0.7	0.7	2.0	-	-
Spathulenol	1568-1576	-			-	-	0.5
Elemol	1549-1572	-	1.3	0.5	0.8	-	
Caryophyllene oxide	1581-1582	1.3	-	9.5	0.6	4.1	4.1
Viridiflorol	1590		-	-	-	0.7	-
5-epi-7-epi- α-eudesmol	1608-1609	0.6	-	-	0.5	-	-
10-epi- γ-eudesmol	1619-1627	3.8	-	-	-	-	-
β- atlanol	1633	-	-	-	-	0.5	-
Epi- α-cadinol	1640-1645-1648	1.2	5.2	-	-	3.4	3.8
Allo-aromadendrene Epoixide	1650-1622-1633	0.9	-	-	-	-	0.7
Epi- α-muurolol	1657-1641-1659	3.6	-	-	-	2.0	2.5
α-muurolol	1645-1662	0.9	-	-	-		-
α- cadinol	1653-1660	-	9.0	-	-	9.9	-
Alloaromadendrene	1461-1664	-	-	-	-	1.2	-
7-epi- α-eudesmol	1658-1685-1687	1.4	8.3	9.5	21.9	-	-
14-hydroxy-9-epi-E-Caryophyllene	1669-1677	-	-	-	-	1.1	-
Cedryl acetate	1762-1763-1766	0.7	2.6	-	1.0	-	1.0
Epi-cyclocoloremone	1769-1771	-	-	-	-	1.4	1.7
14-hydroxy- α-muurolene	1775-1804	2.2	-	-	-	-	-
14-hydroxy- δ-cadinene	1788-1805-1807	-	-	-	-	13.5	15.8

*RI, Retention indices relative to C7-C25 *n*-alkanes on a HP-5MS capillary column

Source of reported chemicals Table 3: (Central Laboratory of Research Institute of Forest and Rangelands, Tehran, Iran).

DISCUSSION

Based on the results of this research, Essential oil obtained from *F. foetida* root sap had a bright red color and a sulfurous pungent odor [27,28]. The butyl propenyl disulphide is responsible for the aroma of *F. foetida* plant [29].

Based on the physiographic characteristics of the six studied habitats related to *F. foetida*, the highest and the lowest average of weight and volume of the sap Essential oil extracted from *F. foetida* root samples were observed from Sabzevar and Dargaz samples, respectively (Table 1). In this direction, Sood reported that the content of essential oil of *F. foetida* is affected by longitude, altitude, and latitude. These factors can directly and indirectly affect the temperature, precipitation, soil, and light and they can change the physical and chemical properties and the volume of the essential oil in *F. foetida* root in different regions [29]. This research showed that there is a negative correlation (r = -73%) between the altitude of the *F. foetida* habitat and the amount of its root sap essential oil (Fig. 2). This result seems to be reported for the first time by us because we could not find a similar report in scientific sources, so this needs further research. The results suggested that the number of chemical compounds of *F. foetida* root sap essential oil belonging to six habitats were almost similar and their number is 24 to 27. We also found 3-5 non-similar compounds in the Anghuzeh root sap oil samples. It seems that the metabolism of *F. foetida* is almost the same in the six habitats [12]. The identified compositions belong to five major chemical groups including terpenes, esters, ethers, organic sulfides, and an organic compound. In this regard, some researchers reported the essential oil compounds of *F. foetida* including terpenes and oxygenated derivatives such as ethers, phenols, alcohols, lactones and esters [2]. In this research, Sesquiterpenes and monoterpenes were the most abundant and esters and thioethers were the least numerous in *F. foetida* essential oil sap (Table 3). the volatile oil that constitutes mono and sesquiterpene components, oxygenated derivatives, thioethers, and esters are the main chemical compounds of *F. foetida* root sap Essential oil. These compounds provide a distinctive flavor, aroma, or scent to a plant [30]. Results showed that terpenoid compounds were the most abundant constituents of *F. foetida* oils (Table 3), however, the essential oils were dominated by volatile Sulphur-containing compounds (Fig. 3, 4, 5, 6, 7 & 8) [30]. As it is observed, z-propenyl-sec-butyl disulfide and e-Propenyl-sec-butyl disulfide in the composition of *F. foetida* root sap essential oil were the highest amount of all for the samples related to Bajestan, Kashmar, Sabzevar and Nishabour, but they were not much in the sample of Kalat (Fig. 8). This is even though that the amount of the mentioned compounds may be different in other regions. The reason for the changes in the composition of *F. foetida* essential oil can be related to altitude, which is the most effective environmental factor and can explain the high levels of variation in the essential oil yield and components in other regions [2]. According to the results obtained from this research, the highest amount of e-propenyl-sec-butyl disulfide in the essential oil of *F. foetida* root sap was in Sabzevar sample (32.12%) and the lowest amount of this compound was found in Kalat sample (0.58%) (Fig. 8). Some researchers reported that the composition of *F. foetida* essential oil is different in root, leaf and seed parts, and it can change quantitatively and qualitatively in climatic conditions, height and growth stage [32]. Also, we found some chemical compounds that were unique to the essential oil of one habitat (Table 3 and Fig. 9). This may be related to edaphic and climatic factors of the habitat or genetic factors [28]. Overall, although the results of this research showed that the height of the habitat has an effect on the amount of *F. foetida* root essential oil but this environmental factor could not show a clear trend in relation to the number of chemical compounds in the essential oil of *F. foetida* root and these results should be applied in the cultivation of this species.

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