Variation in essential oil components among Iranian *Ferula assa-foetida* L. accessions

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**ABSTRACT**

Oleo gum resin is extracted from the *Ferula assa-foetida* L. and is important for pharmacological and industrial applications. In the present study, the essential oil components of fourteen Iranian and Afghan *F. assa-foetida* accessions were analyzed. A total of 20 g of the oleo gum resins were hydro-distilled in a Clevenger type apparatus for 3 h in three replications. The amounts of essential oil yield ranged from 2.53 to 20.85%, indicating a high level of variation in this feature among the accessions. The results of GC-MS analysis discovered 42 compounds in the accessions. Six of these compounds were found most abundantly in the samples. These were (E)-1-propenyl sec-butyl disulfide (13.66–49.35%), β-pinene (1.06–21.18%), (Z)-1-propenyl sec-butyl disulfide (2.02–15.29%), α-pinene (2.04–17.61%), thiophene (0.03–36.81%), and thiourea (0.08–9.63%). Based on cluster analysis, four chemotypes were introduced among the *F. assa-foetida* accessions. The results indicated that as the altitude increased, the amount of β-pinene increased too, whereas the amounts of (Z)-1-propenyl sec-butyl disulfide and thiourea decreased. In addition, the t-test and the correlation analysis revealed that altitude was the most effective environmental factor which explained the high levels of variation in the essential oil yield and components.

1. Introduction

The genus *Ferula* belongs to the Apiaceae family and comprises about 170 species worldwide, of which 30 species have been recognized in Iran (Zomorodian et al., 2018). *Ferula assa-foetida* L. (asafoetida) is an important perennial medicinal herb which is native to Iran and central Asia. Its local names are “koma” and “anghuzeh” in Persia (Samadi et al., 2016; Zomorodian et al., 2018). It is a monoecious and herbaceous plant that gives off an unpleasant odour. The plant grows up to 1.5 to 2 m, and occurs in two forms of taste, i.e. bitter and sweet (Iranian Herbal Pharmacopoeia, 2002; Malekzadeh et al., 2018). Asafoetida is a dried latex (oleo-gum-resin; OGR) which exudes from the roots of some species of the genus *Ferula* (Moghadam and Farbadi, 2015). Extracting the OGR from this plant is important for pharmacological and industrial applications. Asafoetida is characterized by multiple pharmaceutical properties, such as antimicrobial, antibacterial, antispasmodic, anti-inflammatory and muscle relaxant (Kavosoi and Rowshan, 2013; Samadi et al., 2016; Valencia et al., 1994). The OGR of *F. assa-foetida* contains three main chemical compounds, including 10–17% volatile oils (consisting of sulfur-containing compounds and other terpenoids), 25% gum (including glucose, galactose, L-arabinose, rhamnose, glucuronic acid, polysaccharides, and glycoproteins) and 40–64% resin (consisting of coumarins and sesquiterpene coumarins, ferulic acid, and other terpenoids; Amalraj and Gopi, 2017; Takeoka et al., 2001). Essential oils (EOs) are fragrant compounds produced by different parts of plants such as roots, shoots, flowers and buds. The components of EOs include terpenes, sesquiterpenes, and oxygenated derivatives such as ethers, phenols, alcohols, lactones and esters (Abhari et al., 2018; Kavosoi and Rowshan, 2013; Nerio et al., 2010). It has been reported that the EO yield and components often vary among accessions. These variations are highly related to genetic, climatic and soil conditions, altitude, growth phase (i.e. reproductive or vegetative) and different plants parts (Bakkali et al., 2008; Kavosoi and Rowshan, 2013; Najafabadi et al., 2017; Soihi et al., 2019; Solórzano-Santos and Miranda-Novales, 2012). Therefore, valuable information can be obtained by comparing compounds in the EOs of medicinal plants that...
originate from different areas. To date, there have been limited amounts of information on the chemical composition of essential oils extracted from Iranian *F. assa-foetida* accessions, and the variations that may exist in those compositions. Identifying accessions with high-quality EOs may offer opportunities for future breeding efforts. The present study aimed at evaluating the EO yield and compounds of *F. assa-foetida* accessions that were collected from different parts of Iran.

2. Materials and methods

2.1. Plant materials

The OGRs of thirteen Iranian and one Afghan asafetida were collected from eight provinces in Southern, Southeastern, Eastern and central Iran, as well as western Afghanistan, in August 2018 (Fig. 1).

2.2. Isolation of essential oil

The OGRs were dried at room temperature to extract the EOs. A total of 20 g of OGRs were hydro-distilled in a Clevenger type apparatus for 3 h in three replications. The essential oil yield (%) was determined as the volume of EO per 20 g of weight. The EOs were kept at 4 °C in the dark prior to analysis.

2.3. Determination of essential oil components

Essential oil components were detected using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC/MS) methods. The GC analysis of EOs was performed on a Thermoquest-Finnigan model trace GC, equipped with a DB-5 fused-silica column (30 m × 0.25 mm i.d., film thickness × 0.25 μm). The oven temperature was programmed from 60 to 250 °C at a rate of 4 °C min⁻¹ and nitrogen served as the carrier gas. The temperatures of 250 and 280 °C were used for the injector and the detector, respectively. GC–MS was carried out on a Thermoquest-Finnigan model trace GC/MS, equipped with a DB-5 fused-silica column (60 m × 0.25 mm i.d., film thickness × 0.25 μm). The oven temperature was programmed from 60 to 250 at 4 °C min⁻¹ using helium as the carrier gas (1.1 ml min⁻¹). The operating conditions of MS were as follows: The ion source and transfer-line temperature were set to 250 °C, the ionization energy was 70 eV and the mass scan was 40–460 amu. In order to detect the EO components, the retention index (RI) was considered under programmed temperature conditions for n-alkanes (C₆-C₂₄). The RI was compared with the internal reference MS library (Wiley 7.0) and with published data in the literature (Adams, 2007).

2.4. Statistical analysis

A completely randomized design with three replications was applied, and the means of the essential oil percentage were compared using the LSD test (least significant difference) at the 0.01 probability level. Cluster analysis was applied using Euclidean distance as a measure of similitude and by the unweighted paired group average (UPGMA) method. The Cluster and PCA analyses were accomplished.
using SPSS software v21 (SPSS Inc., Chicago, IL) and by the statistical analysis system (SAS) computer software (SAS Institute Cary, NC, USA, 1988), respectively. The correlations were visualized as a coloured heat map using MetaboAnalyst (Xia and Wishart, 2016).

3. Results and discussion

3.1. Essential oil yield

The EO yields of the plants were presented in Fig. 2. The results of ANOVA showed a significant difference among the accessions for this character \( P < 0.01 \); data not shown). The highest (20.85%) and the lowest (2.53%) EO yields in OGRs were obtained in Neyriz and Yasuj accessions, respectively. Hadavand Mirzaei and Hasanloo (2014) reported 2.3 and 2.1% EO yields extracted from OGRs of Tabas and Yazd samples, respectively. The aerial parts of asafoetida yielded 1.7% in Neyshabour accession (Samadi et al., 2016). In addition, the EO yields of \( F. \) assa-foetida in the leaves and fruits were 0.043 and 0.49%, respectively (Bamoniri and Mazoochi, 2009). The EO yields in OGRs from Isfahan, Kerman, and Lar were 6.5, 6.03, and 8.75%, respectively (Nazari and Alizadeh, 2014; Sadraei et al., 2003). The extraction of EO from OGRs at different times of the year, i.e. 15 and 30 June as well as 15 July, yielded different levels of EO that amounted to 9, 6, and 4%, respectively (Kavossi and Rowshan, 2013). According to the literature review, the EO yield in previous studies ranged from 0.043 to 9%. The geographical origin, harvest time and plant parts were recognized as the main causes of variation in this range of percentages. However, the EO yield in the present study ranged from 2.53 to 20.85%, indicating a high level of variation in this character among the studied accessions. Genotype and environment are the main factors of how plants store and apply resources and influence the production of the EOs and compounds, which is often increased by different biotic and abiotic stresses (Farajpour et al., 2017; Langenheim, 1994). Therefore, environmental and genetic differences can be the causes of this variation in the EOs of Iranian \( F. \) assa-foetida accessions.

To classify the EO yields of plants, the accessions were divided into four groups based on the cluster analysis (Fig. 3). According to Fig. 2, the highest values of EOs were obtained in the second and third groups. The accessions of these groups were collected from a higher altitude compared with the two other groups. The correlation analysis was performed to find a relationship between different environmental factors, including the average annual precipitation and temperature, and altitude with essential oil yield (Fig. 4). The results of the correlation analysis showed a positive moderate correlation between essential oil yield and altitude \( r = 0.45; \quad P < 0.1 \). Previous studies showed relationships between altitude and EO yield in different plants such as \( Coriandrum sativum \) (Shams et al., 2016), \( Artemisia rupestris \) (Haidar et al., 2009), and \( Achillea aucheri \) (Sardorodi et al., 2017).

3.2. Essential oil composition

The compounds of EOs that were extracted from the \( F. \) assa-foetida accessions were detected using GC-MS analysis (Table 1). A total number of 42 compounds were recognized in the samples. A large number of these compounds were generally detected in all of the accessions. Accordingly, the main constituent of the EOs was \( (E)-1\)-propenyl sec-butyl disulfide (13.66–49.35%), followed by \( \beta \)-pinene (1.06–21.18%), \( (Z)-1\)-propenyl sec-butyl disulfide (2.02–15.29%), \( \alpha \)-pinene (2.04–17.61%), thiophene (0.03–36.81%), and thiourea (0.08–9.63%). Previous studies reported that the important compounds in the EO of OGR extracted from \( F. \) assa-foetida were \( (E)-1\)-propenyl-sec-butyl disulfide (22.1–55.64%), \( (Z)-1\)-propenyl-sec-butyl disulfide (3.54–35.1%), \( \alpha \)-pinene (2.28–21.3%), \( \beta \)-pinene (3.4–47.1%), epi-\( \alpha \)-Cadinol (23.15%), 1,2-Dithiolane (18.6%) glubolol (12.5%), germa-crene B (10.98%), germacrene B (7.8%), \( \alpha \)-gurjunene (6.18%), \( (Z)-\beta \)-Ocimene (4.96%), and germacrene D (3.09%) (Delavar et al., 2014; Khajeh et al., 2005; Nazari and Alizadeh, 2014; Sadraei et al., 2003; Yousefi et al., 2011). According to previous reports and the results of this study, there was a high level of variation in the components of Iranian \( F. \) assa-foetida accessions. These variations can be sourced from many factors such as genetic variation among the accessions, different plant parts, geographical origin, climatic variation, as well as the extraction, drying and storage methods (Boroomand et al., 2018; Ebrahimim et al., 2012; Rajabi et al., 2014). In the present study, six compounds were selected as the most prominent constituents of the EO, based on the average of each compound among all accessions. However, some individual components were observed in several accessions only. These compounds were \( \delta \)-seline in Lar, Safashahr, and Boshrouyeh accessions (9.33, 7.02, and 6.25%, respectively), allo-ocimene in Shahrbabak, Safashahr, Lar, and Yasuj accessions (7.73, 6.91, 5.81, and 5.25%, respectively) and guaiol in Lar and Safashahr accessions (4.51 and 3.47%, respectively).

3.3. \( F. \) assa-foetida chemotypes

A dendrogram was generated using the top six main compounds (Fig. 3). According to cluster analysis, the Iranian \( F. \) assa-foetida accessions were classified into four groups. The first group consisted of Tabas and Boshrouyeh accessions. These two accessions were found to be rich in thiophene (36.63 and 36.81%, respectively), while they had the lowest percentage of \( \beta \)-pinene and \( (E)-1\)-propenyl sec-butyl disulfide, compared with other accessions. The second group contained three accessions, i.e. Shahrebabak, Yasuj, and Zarand. This group was rich in \( \beta \)-pinene and \( (E)-1\)-propenyl sec-butyl disulfide. Accordingly, higher percentages of \( (E)-1\)-propenyl sec-butyl disulfide were observed in the Yasuj, Zarand, and Shahrebabak accessions (49.35, 48.34 and 40.11%, respectively). Neyriz, Zahedan, Marvdasht and Safashahr accessions formed the third group, which had high levels of \( \alpha \)-pinene, \( \beta \)-pinene, and \( (E)-1\)-propenyl sec-butyl disulfide components. The percentages of \( \alpha \)-pinene in these accessions were more than the others. The fourth group consisted of five accessions, namely the Naian, Ferdows, Lar, Jiroft, and Afghanistan accessions. These accessions were rich in \( (Z)-1\)-propenyl sec-butyl disulfide and \( (E)-1\)-propenyl sec-butyl disulfide components. The highest percentages of thiourea (11.41%) and \( (Z)-1\)-propenyl sec-butyl disulfide (15.29%) were observed in Ferdows and Afghanistan accessions, respectively.

Based on the percentage of each compound in the groups, four chemotypes were introduced for the Iranian \( F. \) assa-foetida accessions.

i. Thiophene (36.72%; 2 accessions). 
ii. \( \beta \)-pinene (16.29%) + \( (E)-1\)-propenyl sec-butyl disulfide (45.93%; 3 accessions). 
iii. \( \alpha \)-pinene (13.69%) + \( \beta \)-pinene (16.06%) + \( (E)-1\)-propenyl sec-butyl disulfide (27.05%; 4 accessions). 
iv. \( (Z)-1\)-propenyl sec-butyl disulfide (11.53%) + \( (E)-1\)-propenyl sec-
butyl disulfide (33.94%; 4 accessions).

There are many factors that control the quantity and quality of phytochemical components in each medicinal plant. These factors include, but are not limited to, physiological variation, genetic and environmental factors, soil conditions, different plant parts and geographical origin, as well as the extraction, drying and storage methods. However, many factors remain as unknown, and the ones that are already known have not received considerable amounts of attention for research (Farajpour et al., 2017; Figueiredo et al., 2008). In this study, it was endeavored to find a relationship between the four groups with the environmental factors including average annual temperature and precipitation and altitude. To this end, an unbalanced completely randomized design was used, whereupon each group was considered as a treatment and its accessions were the replications. The results showed that there was a significant difference among the groups with respect to altitude ($P < 0.05$), while no significant difference was observed for the other two environmental factors. In addition, the results of mean comparison showed that there were neither significant differences between the first and the fourth groups, nor between the second and the third groups, whereas the first and the fourth groups were significantly different from the other two groups. Therefore, in order to find a relationship between the phytochemical components and altitude, the $t$-test was used for comparing accessions of the low altitude treatment (belonging to the first and the fourth groups) with those of the high altitude treatment (belonging to the second and the third groups). The $t$-test showed that $\beta$-pinene and ($Z$)-1-propenyl sec-butyl disulfide components differed significantly because of the altitude treatments ($P < 0.01$). According to the correlation analysis, $\beta$-pinene ($P < 0.01$), ($Z$)-1-propenyl sec-butyl disulfide and thiourea correlated significantly with altitude (Fig. 4). The accessions of the first group inhabited a lower altitude compared to other accessions. These accessions were also rich in thiophene, indicating that this component is affected by altitude. According to the results of the $t$-test and correlation analysis, the increase in altitude was parallel to the increase in $\beta$-pinene, but the decrease in ($Z$)-1-propenyl sec-butyl disulfide and thiourea. Nasiri Bezenjani et al. (2017) studied the correlation between environmental factors with EO components of $F$. assa-foetida. The authors reported that altitude correlated significantly with tannin (-0.87), total phenolic content (0.63) and ferulic acid content (0.56). At higher altitudes, there were lower amounts of $\alpha$-thujone and higher amounts of chamazulene (a harmful and a valuable components, respectively; Farhang-Sardrodi et al., 2017). Fig. 4 also shows the correlations among the top six constituents. The results indicated that the highest correlation coefficient was between thiophene and ($E$)-1-propenyl sec-butyl disulfide compounds ($r = -0.71; P < 0.01$). In addition, thiophene had significant correlations with $\beta$-pinene and thiourea constituents ($P < 0.05$). Meanwhile, $\alpha$-pinene had a positive significant correlation with

Fig. 3. Dendrogram of 14 $F$. assa-foetida accessions, according to the top six compositions using UPGMA method.

Fig. 4. Heat map of the correlations among the top six components of $F$. assa-foetida and environmental factors. * and ** indicate significance at 5 and 1% levels, respectively. Z and E are ($Z$)-1-propenyl sec-butyl disulfide and ($E$)-1-propenyl sec-butyl disulfide components, respectively.
Table 1

<table>
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<tr>
<th>Component</th>
<th>R²</th>
<th>R²p</th>
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<th>2</th>
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<th>13</th>
<th>14</th>
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<td>0.26</td>
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<td>0.24</td>
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<td>3.1</td>
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<td>3.5</td>
<td>11.4</td>
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<td>2.25</td>
<td>3.12</td>
<td>0.25</td>
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<td>a</td>
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<td>0.25</td>
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<td>0.11</td>
<td>t</td>
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<td>0.38</td>
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<td>0.19</td>
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<td>t</td>
<td>a,b</td>
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<td>t</td>
<td>a,b</td>
<td></td>
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</tr>
<tr>
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<td>0.63</td>
<td>0.82</td>
<td>0.98</td>
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</table>

Principal component analysis (PCA) was performed based on the top six essential oil compounds of 14 Ferula assa-foetida accessions. The first two principal components (PC1 and PC2) explained more than 94% of the total variance among the studied accessions (Table 2). The PC1 confirmed 64% of the total variance and correlated positively with (E)-1-propenyl sec-butyl disulfide (0.97), α-pinene (0.93), (Z)-1-propenyl sec-butyl disulfide (0.88), and α-pinene (0.82). Thiophene and thiourea components showed a positive correlation with the second PC. The PC2 explained more than 30% of the total variance. Therefore, environmental factors can primarily explain the variation among the different accessions of Ferula assa-foetida that were collected from different regions of Iran. Based on the t-test and the correlation analysis, altitude was found to be the most effective environmental factor.

Table 2

| Label Major compound Principal components |
|------------------------------------------|------------------------------------------|
| (E)-1-propenyl sec-butyl disulfide       | 0.97                                      |
| β-Pinene                                 | 0.93                                      |
| (Z)-1-propenyl sec-butyl disulfide       | 0.88                                      |
| α-Pinene                                 | 0.82                                      |
| Thiophene                                | 0.55                                      |
| Thiourea                                 | 0.54                                      |
| Eigenvalue                               | 3.84                                      |
| % of variance                            | 64                                        |
| Cumulative%                              | 94.45                                     |
4. Conclusion

In the present study, 13 Iranian *F. assa-foetida* accessions were evaluated with respect to the phytochemical components of their EOs. The amounts of EO in different accessions ranged from 2.53 to 20.85%, indicating a high level of variation among the accessions. According to the GC-MS analysis, the top six constituents of EO in the samples were (E)-1-propenyl sec-butyl disulfide, β-pinene, (Z)-1-propenyl sec-butyl disulfide, α-pinene, thiphenole, and thiourea. Based on cluster analysis, four chemotypes were introduced among the *F. assa-foetida* accessions. The results indicated that with an increase in altitude, the β-pinene increased, whereas the (Z)-1-propenyl sec-butyl disulfide and thiourea decreased. It can be concluded that environmental factors explained the high levels of variation in the EOs and in their components, which altitude was found to be the most effective factor.

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