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Chemical composition of essential oil andantioxidant activity of Iranian and Afghanestanian population of *Ferula assa-foetida* L

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ABSTRACT

In this study, the essential oil composition, antioxidant activities, phenolic and flavonoid contents, and of metanolic extract of Iranian and Afghanestanian populations of *Ferula assa-foetida* were evaluated. Essential oils were analyzed by using GC and GC/MS, The antioxidant activity were measured by 2, 2-diphenyl-1-picrylhydrazyl and β-carotene/linoleic acid assay, also phenol and flavonoid content were measured by gallic acid and quercetin as standard compound. Result showed that propenyl sec butyl disulfide (42.72%), acetone, dimethyl mercaptole (14.84%) and gamma- eudesmol (8.6%) were the main compouned found in the Iranian population while; the Afghanestanian population were rich of propenyl sec butyl disulfide (39.61%), acetone, dimethyl mercaptole (18.25%) and 8-ethyl-pentathiadecane (18.97%). In DPPH and β-carotene/linoleic acid tests, Iranian and Afghanestanian populations have stronger antioxidant activity respectively, also, the results demonstrated that the Afghanestanian population have more phenolic and flavonoid contents than the Iranian population. Overall, the results showed that the two populations investigated in this research have differences in terms of the type and percentage of essential oil compounds, antioxidant activity and phenol and flavonoid content. These differences are probably caused by differences in ecological factors such as the habitats of these populations and the influence of these factors on the studied parameters.

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1-Introduction

Medicinal plants have been used by humans for centuries to treat various diseases in traditional medicine. In recent years, due to the toxicity and side effects of some synthetic drugs, the use of drugs of natural origin has accelerated [1]. The Secondary metabolites of medicinal plants, including phenolic compounds that are present in different organs of the plant such as leaves, stems, roots, etc., by preventing oxidation and spoilage, increase the quality of food and increase their useful life [2]. Studies show that the activity of free radicals in the body can provide the basis for the occurrence of cancer and cardiovascular diseases. The use of plants with antioxidant properties can be an important for scavenging of free radicals [3]. In general, it can be said that cheap price, non-toxicity and effect at low concentrations are the most important features of plant antioxidants, which has caused a significant demand by food and pharmaceutical factories to use plant derivatives as antioxidants in food systems [4, 5]. Ferula assa-foetida is a monocarpic plant with the scientific name of *Ferula assa-foetida*. It has a straight, fleshy and relatively thick root that stores a sap called Ferula. This plant is native to Central Asia from eastern Iran to Afghanistan [6]. Helping to treat various diseases such as digestive disorders, nervous disorders, respiratory problems and treating insect bites is one of the most important uses of *Ferula assa-foetida* in traditional medicine [6]. *Ferula assa-foetida* plant is one of the most important and characteristic medicinal plants (especially in Afghanistan) which is used in different ways (raw, decoction, extract, powder, etc.) to treat various diseases such as: stomach pain, sexual enhancement, hemorrhoids, problems It is used for menstruation, colds, epilepsy, antioxidant properties, fungicidal, bactericidal, gout, etc.

In various studies, the components of the essential oil of different species of *Ferula assa-foetida* h have been investigated. In all these studies, sulfur compounds have been introduced as the main constituents of *Ferula assa-foetida* essential oil. The results of Bahrami et al.'s study in 2013 show that E-1-propenyl sec-butyl disulfide and Z-1 propenyl sec-butyl disulfide and Z-1 propenyl sec-butyl disulfide are the most abundant components of *Ferula assa-foetida* essential oil. is [7]. The investigations carried out by Nasiri Berjani et al. in 2016 led to the identification of 51 compounds in the essential oil of Ferula assa-foetida L., which are 1-propenyl sec-butyl disulfide, n-propyl sec-butyl disulfide, Z-beta osimen and Beta-pinene was introduced as the main component of the essential oil of this species [8]. Nazari and Iranshahi reports in 2011, E-1-propenylsec-butyl disulfide and Z-1-propenylsec-butyl disulfide, alpha-pinene, beta-pinene, guayol and caratol as the most important constituents of F. assa gum. -foetida introduces [9]. The very strong aromatic smell of the essential oil of Ferula species, which is usually obtained from different parts of the plant, including flowers, seeds, leaves, stems and roots, is due to the presence of volatile sulfur compounds. In recent years, several pharmacological effects have been suggested for these volatile compounds, which can be mentioned as anticancer, antioxidant, blood lipid lowering, antibacterial, neuroprotective, and immune modulating effects [7].

The suitable weather conditions of the eastern regions of Iran and Afghanistan for the cultivation and exploitation of the *Ferula assa-foetida* plant, which is now at risk of extinction, is considered as a potential capacity for the development of agriculture in these regions. In this research, our goal is to identify and compare the constituents of the essential oil and investigate the antioxidant effects of two Iranian (Khorasani) and Afghanestanian

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Ferula assa-foetida populations. The results of this research can help farmers to choose the right population for its cultivation and exploitation

The products obtained from this plant are helpful.

2-Materials and methods

2-1Plant material

Iranian gum or leachate was collected from the eastern population of Iran and from Khorasan Razavi province, while the Afghanestanian population was produced from samples cultivated in Balkh province, Afghanistan.

2-2 Essential oils extraction

The essential oils were extracted by hydrodistillation using Clevenger type apparatus. Distillation process was done for 3 h. The obtained essential oils were stored in the freezer at −20°C until analysis.

2-3 Analysis of essential oils

Flame ionization detector-gas chromatography (FID-GC) was performed using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d., 0.25 µm film thickness); carrier gas, He; split ratio, 1:25, and FID. Temperature program: 60°C (2 min) rising to 240°C at 4°C/min; injector temperature, 250°C; detector temperature, 260°C. GC-MS was performed using Hewlett-Packard 6859 with quadrupole detector, on a HP-5 column (GC), operating at 70 eV ionization energy, using the same temperature program, and carrier gas as mentioned earlier. Retention indices were calculated by using retention times of n-alkanes that were injected after the oils at the same chromatographic conditions according to Van Den Dool's method Identification of the components was done by comparing their mass spectra with those of internal Wiley Gas chromatography-Mass Spectrometry (GC-MS) spectral library, or with published mass spectra and those described by Adams [10].

2-4 Preparation of extracts

5 grams of the crushed sample was poured into a bag with fine holes. The bag was placed inside a glass container with a lid and methanol was poured on it until the surface of the bag was completely covered. At the end of the work, after closing the glass lid and wrapping the aluminum foil around the containers, the containers containing the extract were transferred to a dark place. After three days and nights (72 hours), the extracted extract was filtered and transferred to -4°C refrigerator and added again to the original container containing the solvent bag. and then all the described steps were repeated. In total, during 9 days and nights, adding the solvent and filtering the extract was done three times. The desired extract was filtered with filter paper, then the concentration was done using the rotary device of IKA company model RV06-ML and vacuum pump STEROUAO.

2-5 Investigating the antioxidant activity of different extracts

2-5-1 DPPH assay

In this spectrophotometric method, DPPH free radicals are used as a reagent, and the activity of hydrogen atoms and electrons of the extracts is measured through the discoloration of the deep purple DPPH solution. From the dried extracts, solutions with concentrations of (0.04, 0.03, 0.02, 0.01 and 0.005) mg/ml were prepared. Then, in order to check the antioxidant property, a 0.004% solution of DPPH was prepared (the resulting solution is deep purple in color), then three test tubes were prepared for each sample, and 50 microliters of the extract and one milliliter of DPPH solution were added to each tube. Next, the tubes were transferred

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to a dark place and after 30 minutes of greenhouse at room temperature, the absorbance of the samples was read at a wavelength of 517 nm. In this method, BHT was used as a positive control and methanol as a negative control. Using the following formula, the inhibition percentage was calculated.

I% = (Ablank – Asample/Ablank) × 100

In this regard, Ablank is the absorption of the negative control reaction (including all reagents, except the defined concentration of the given extrac) and Asample is the absorption of the desired sample in the reaction mixture (19). In the following, using the graph obtained from the amount of I, the final results were expressed as IC50 (expressing a concentration of the extract that causes 50% inhibition of oxidative processes) in terms of micrograms per milliliter [11].

2-5-2 β-carotene/linoleic acid assay

In this method, the antioxidative potential of the extracts was measured by plotting decolorization of the β-carotene/linoleic acid assay. To prepare the β-carotene/linoleic acid solution, 0.5 mg β-carotene was mixed with 1 mL chloroform, and then 25 µL linoleic and 200 mg Tween-40 were added. The chloroform was completely evaporated. In the next stage, 100 mL oxygen-saturated distilled water was added and the container was vigorously shaken. A solution with a concentration of 1 mg/ml was prepared from the extract of each population. To check the antioxidant property, 50 microliters of the extract was added to 2500 microliters of beta-carotene-linoleic acid solution.. In zero time and after 2 h incubation at 50°C, the absorbance of the specimens were measured at 470 nm using a microplate reader (Bio Tek, USA). The antioxidative capacity of the extract was compared with positive tests. All the tests were carried out in triplicate. The activity was expressed as inhibition percentage using the following equation: AA% = (1 – DRS/DRC) × 100

where AA% is the antioxidant activity, DRC and DRS are the degradation rates of β-carotene in reactant mixture without and with the sample, DR = ln (a/b) × 1/t where a = initial absorbance at 0 min, b = absorbance at 120 min, and t = 120.[1]

2-6 Phenolic compounds

In order to measure phenolic compounds, Folin-Ciocaltue was used as a reagent and gallic acid was used as a standard. A solution with a concentration of 2 mg/ml was prepared from the extract of each population, and solutions with different concentrations were prepared from gallic acid. Three test tubes were prepared for each solution and 100 microliters of extracts or gallic acid in each tube was mixed with 1500 µL of Folin-Ciocalteu reagent (diluted ten-fold) and 1 mL distilled water was added; after 1 min, 1500 µL of a solution of 20% sodium carbonate was added and the mixture was kept in the dark at room temperature, then absorbance was measured at 760 nm was read using a microplate reader [12], finally, the amount of phenol in the extracts was reported in micrograms of gallic acid per milligram of dry weight of the extract using the equation obtained from the standard graph.Absorbance :0.0021 Gallic acid (µg) +0.0022 (r^2 = 0.987)

2-7 Flavonoid compounds

In order to measure flavonoid compounds, aluminum chloride was used as a reagent and quercetin as a standard. From the extract of each population, solutions with a concentration of 2 mg/ml were prepared, and from quercetin, solutions with different concentrations were prepared, then three test tubes were prepared for each solution. Here, 500 µL was mixed with 1500 µL methanol,

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100 µL of 10% aluminum trichloride, 100 µL of potassium acetate 1 M, and 2.8 mL of distilled water. After 40 min at room temperature, the absorbance was determined at 420 nm using a microplate reader [13]. The same procedure was repeated for all standard quercetin solutions and the concentration of flavonoid compounds was calculated accordingly and the standard curve was obtained using the following equation:

Absorbance: 0.0091 quercetin (µg/ml) + 0.0206 (r^2 = 0.995)

2-8 Statistical analysis

Experimental results were represented as mean ± standard error (SE) of three parallel measurements and analyzed by the Minitab software. Differences between means were determined using Tukey's and student's-t-test.

3 Results

3-1 Chemical composition of essential oils

The results of the essential oil analysis of Iranian and Afghanestanian *Ferula assa-foetida* populations are shown in table (1). Based on the results of this table, propenyl sec butyl disulfide (42.72%), acetone, dimethyl mercaptole (14.84%) and gamma-eudesmol (8.6%) are the key compounds found in Iranian essential oil. In the Afghanestanian population, propenyl sec butyl disulfide (39.61%), acetone, dimethyl mercaptole (18.25%) and 8-ethyl-pentathiadecane (17.97%). are the most important components of essential oil. The results obtained from the analysis (GC-MS) show that each of these two populations of *Ferula assa-foetida* has its own unique compounds, but at the same time, some key essential oil compounds (propenylsecbutyl disulfide and acetone dimethylmercaptol) in two The study population is common. The amount of propenylsecbutyl disulfide in the Iranian population is 7.28% higher than that of the Afghanestanian population (Figure 1). While the amount of dimethyl mercaptol in essential oil in the Afghan population is 20.66% higher than that of the Iranian population (Figure 2).

Table 1. Essential oil composition of Iranian and Afghanestanian populations of *Ferula assa-foetida*

		Area %	
Compounds	**RI**	Iranian population	Afghanestanian population
alpha.-pinene	939.54	3.88	0.10
Ethylbenzene	956.42	-	0.04
Camphene	961.19	0.10	-
beta.-Pinene	1004.53	2.38	-
2,3,4-Trimethylthiophene	1022.62	0.11	-
trans-Ocimene	1046.10	1.49	-
Cis-.beta.-ocimene	1068.32	3.01	-
Acetophenone	1098.62	-	0.13
1-methylpropyl disulfide	1310.85	-	0.92

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fenchyl acetate	1312.35	-	0.1
allyl nicotinate	1314.51	-	6.07
3-Methyl-4,5-dithiaoctane	1274.51	1.21	-
propenyl sec butyl disulfide	**1293.33**	**42.72**	**39.61**
acetone, dimethyl mercaptole	**1441.61**	**14.84**	**18.25**
8-ethyl-4,5,6,7,9-pentathiadecane	1461.09	-	17.97
beta.-eudesmene	1500.61	0.60	-
2,3-dimethyl-3-hexanol	1510.61	-	1.04
dihydro-.beta.-agarofuran	1517.76	3.83	-
Guaiol	1607.13	1.51	-
alpha.-eudesmol	1619.19	0.91	-
gamma.-eudesmol	3635.79	8.6	-
Agarospirol	1648.55	1.4	-
Torreyol	1666.56	1.87	-
Total		88.46	84.23

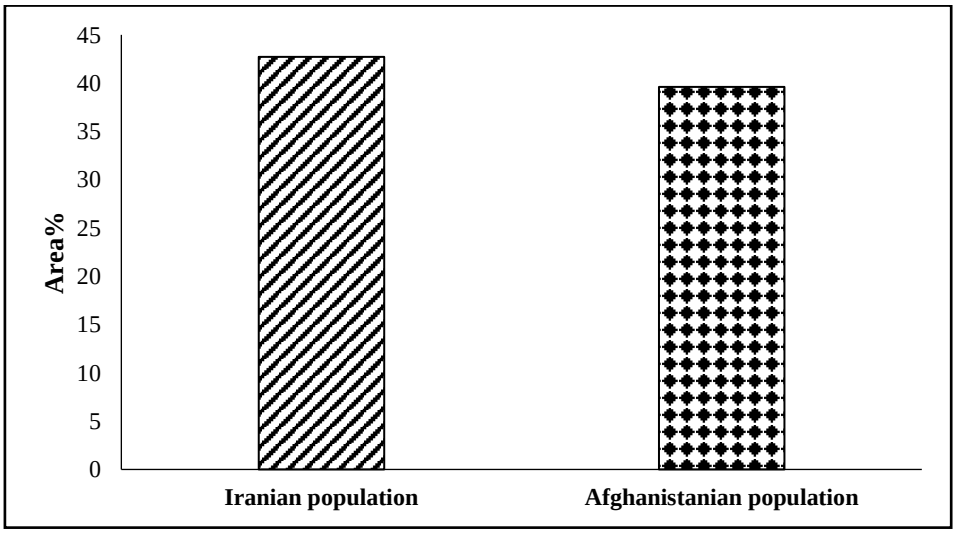

Fig 1. Quantitative changes of propenyl sec butyl disulfide as the first main compound in essential oil of Iranian and Afghanestanian populations.

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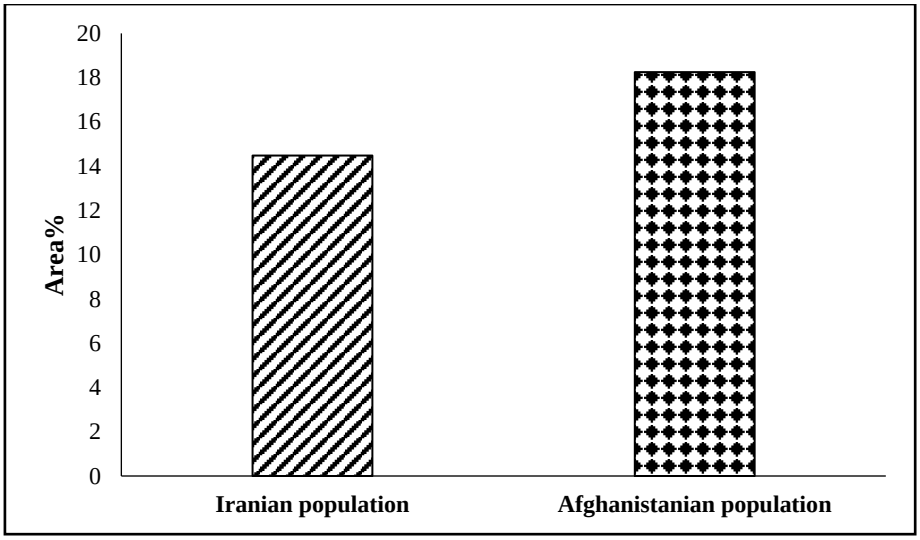

Fig 2. Quantitative changes of Acetone, dimethyl mercaptole as the second main compound in essential oil of Iranian and Afghanestanian populations.

3-2 Investigation of antioxidant activities

3-2-1 DPPH assay

The results of measuring antioxidant activity by DPPH method showed that there is a significant difference between IC50 of Iranian, Afghanestanian populations and BHT at the 5% probability level. Considering that the lower the IC50 value, the higher the antioxidant power, the extract of the Iranian sample has more antioxidant power than the Afghanestanian sample, but the antioxidant power of BHT is higher than that of the Iranian and Afghanestanian populations (BHT > Iranian population> Afghanestanian population) (Figure. 3A).

3-2-2 β-carotene/linoleic acid assay

The results of measuring the antioxidant activity of the methanolic extract of *Ferula assa-foetida* with β-carotene/linoleic acid assay showed that the antioxidant power of Afghan population is higher than Iranian and Iranian population is higher than BHT (Afghanestanian population > Iranian population> BHT) (Figure 3B).

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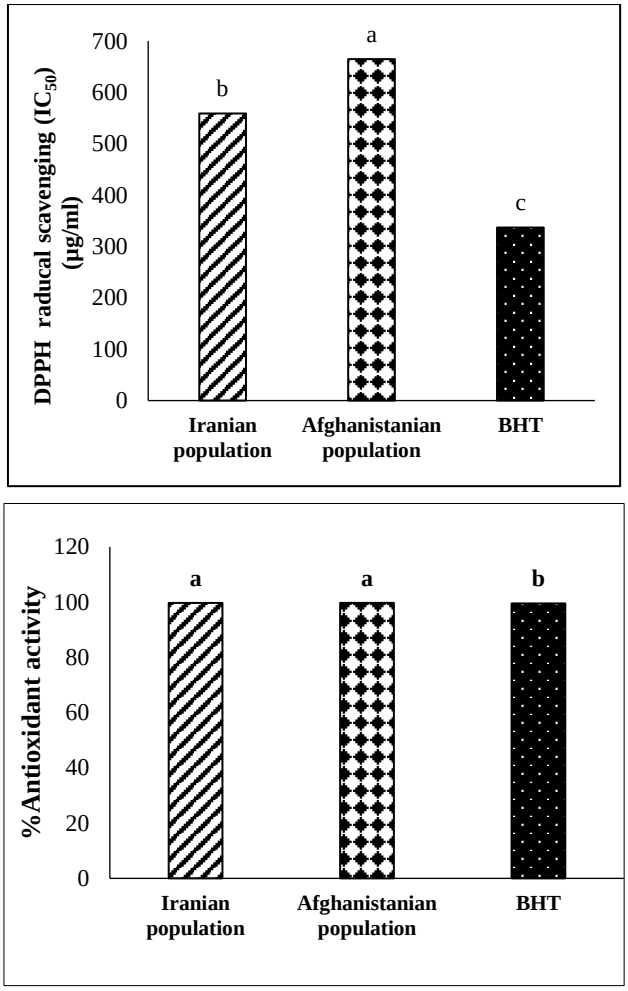

Fig.3. Antioxidant activity of Iranian and Afghanestanian populations of *Ferula assa-foetida* compared with BHT by DPPH (A) and β-carotene-linoleic acid (B) assays. Different letters indicate significant differences at P < 0.05 among treatments.

3-3 Total phenolics

The results obtained from the comparison of the averages showed that at the 5% probability level there is a significant difference between the phenol content in the Iranian and Afghanestanian populations and the amount of these compounds in the Afghanestanian population (251.8 micrograms of gallic acid per milligram of extract) is more than the Iranian population. (1.236 µg of gallic acid per mg of extract) (Figure A4).

3-4 Total flavonoids

The results obtained from the statistical analysis indicate that there is a significant difference between the flavonoid content in the Iranian and Afghanestanian populations. The Afghanestanian population (22.12 µg quercetin/mg extract) has a higher flavonoid content than the Iranian population (19.23 µg quercetin/mg extract) (Figure B4).

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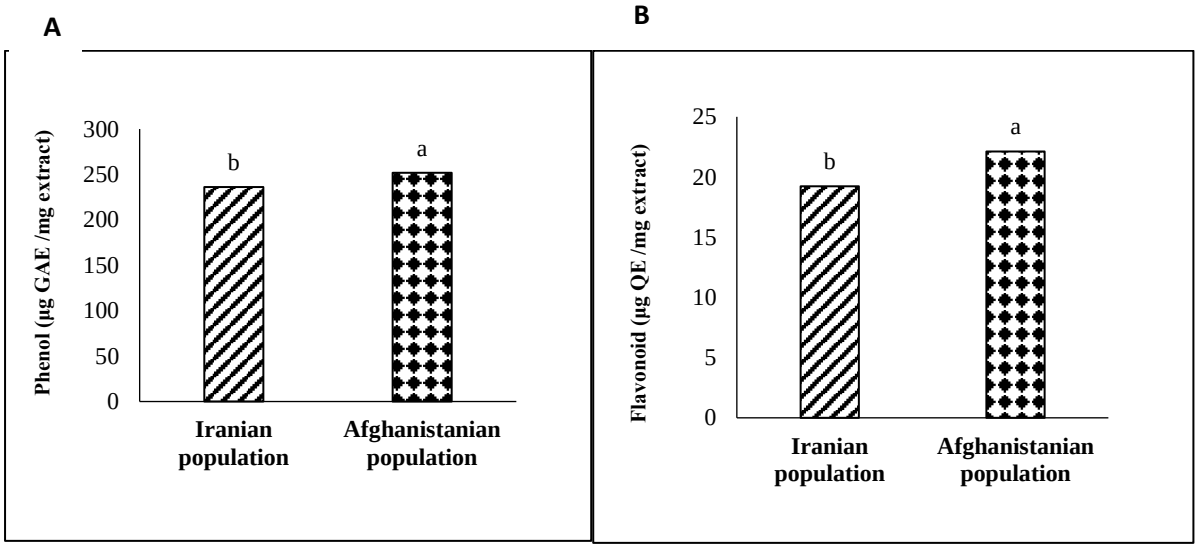

Fig4. Phenol (A) and Flavonoid (B)contents of Iranian and Afghanestanian populations of *Ferula assa-foetida*. Different letters indicate significant differences at $P < 0.01$ among treatments.

4-Discussion

The comparison of essential oil constituents of Iranian and Afghanestanian populations of *Ferula assa-foetida* showed that essential essential oil compounds including propenylsecbutyl disulfide and acetone, dimethyl mercaptole are found in both populations, despite the fact that their amounts show differences in the two studied populations. to give On the other hand, the compound 8-ethyl-9,7,6,5,4pentadecane with a value of 17.9% was identified as one of the key compounds in the essential oil of the Afghanestanian population, while it was not found in the essential oil of the Iranian population. In contrast to this situation, there is a gamma edismol compound in the Iranian population in such a way that this compound is one of the main ingredients of the essential oil of the Iranian population with an amount of 6.8%, while this compound was not detected in the Afghan population. The observed differences in the ingredients of the essential oil can be attributed to the influence of the environmental and ecological factors of the place of growth on the growth and physiological characteristics of the Angouzeh plants studied in this research. In the studies of Nazari and Iranshahi, Khajeh et al., and Amini et al., as in the present study, propenyl-sec-butyl disulfide compound (Figure 7) is mentioned as one of the key components of *Ferula assa-foetida* essential oil [9, 14, and 6]. Investigations conducted on five different populations of *Ferula assa-foetida* in the habitats of Kerman province showed that the most important compound in all regions is propenylsecbutyl disulfide, but its amount is different in different habitats [8], which is with the results of the present study in terms of the effect of the habitat on chemical compounds. essential oil matches. The effect of ecological factors of southwestern Iran habitats on the amount of gum and chemical compounds of the essential oil of the medicinal plant Ferula assa-foetida L. was investigated by Abyar et al. in 2015. The results of their study, similar to the present study, while introducing n-propenyl sec-butyl disulfide as the

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main component of the essential oil of this plant in the different habitats studied, showed that the percentage of the components of the essential oil is different in different habitats. They stated that although secondary metabolites are basically made by directing genetic processes, environmental factors affect their production [15]. Since the sulfur compounds in the essential oil have pharmacological effects such as sedatives and anticonvulsants and are also used in the treatment of rheumatism and diabetes; Therefore, the best quality of essential oil belongs to the population that has the highest percentage of sulfur compounds, and in this study, it seems that the Iranian population is preferable in this regard.

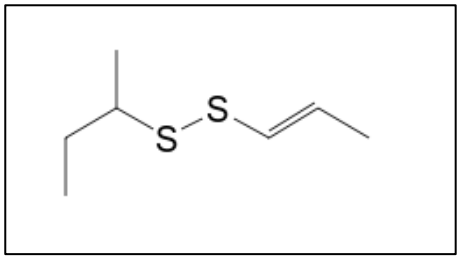

Fig 5. The chemical structure of propenyl sec butyl disulfide as the most important chemical compound of essential oil.

During their life, plants are attacked by many pests and diseases, including herbivores, nematodes, fungi and bacteria. Therefore, to defend themselves, they adopt two mechanisms of mechanical defense (production of spines and hard hairs, silicon bodies, cellulose and lignin) and chemical (production of secondary metabolites such as alkaloids, glucosinolates and phenols) [16]. It seems that the production of sulfur essential oils in *Ferula assa-foetida* plant is related to the chemical defense of the plant against pathogens and herbivores.

Antioxidant activity of plants is mainly due to biologically active compounds, especially phenols. Phenols are structurally composed of an aromatic ring, one or more hydroxyl substituents. The antioxidant activity of these molecules is due to their ability to scavenge free radicals, give hydrogen atoms or electrons, or chelate metal cations [17]. There have been reports that there is a high correlation between the geographical origin of plants and effective substances [18]. Changes in ecological factors such as temperature, rainfall, light intensity and altitude are among the most important environmental factors affecting the formation, quantity and quality of effective substances (secondary metabolites) in plants [19]. The results of this research showed that the antioxidant power of *Ferula assa-foetida* of Iranian and Afghanestanian populations as well as their phenol and flavonoid levels are different from each other. Comparing the results of our research with the results of other researchers, including Ahmadvand et al. and Wahabi et al., shows that the antioxidant power and the amount of phenol and flavonoids in our studied populations are in some cases more and in some cases less than the populations investigated in the studies. these differences show that the type of habitat and the ecological conditions governing it are factors affecting the content of secondary metabolites in plants [20, 21]. In line with our results, Mehrpour et al. in 2015 stated that the type of habitat has an effect on the antioxidant power and the amount of phenol and flavonoid of *Ferula assa-foetida*, so that in their research, the population of Semnan compared to the population of Khorasan had lower IC50 and phenol and flavonoid content. had more [22]. The results of this study are similar to the study conducted by Narimani et al. in 2021, because in this study, the antioxidant power and phenol and flavonoid content in different populations of *Ferula cupularis* showed differences

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[23]. In line with our results, the studies conducted on *Citrullus colocynthis* L in three populations of Kerman, Iranshahr and Zabul showed that the type of habitat is effective on antioxidant power and phenol and flavonoid levels, so that the Kerman population has higher antioxidant power and higher phenol and flavonoid levels in compared with two other populations [24]. The results of this research showed that the antioxidant power and the amount of phenol and flavonoid in two Iranian and Afghanestanian populations are different from each other. In the DPPH method, the Iranian population had a higher antioxidant power, while in the β-carotene/linoleic acid method, the Afghan population showed a higher antioxidant power. These differences are expressed in the amount of phenol and flavonoid and antioxidant power. Probably the type of phenolic compounds (one of the important factors influencing the antioxidant power) is different in these two populations, or possibly other antioxidant compounds such as saponins and carotenoids are the cause of the difference in the antioxidant power of these two populations.

5- Conclusion

The results showed that the type and percentage of chemical compounds that make up the essential oil and the antioxidant power of the extract are different in different populations of *Ferula cupularis*. Since the amount of the important medicinal compound propenyl sec-butyl disulfide in the Iranian population was higher than in the Afghanestanian population; Therefore, it is suggested to use the important and medicinal sulfur secondary metabolites of this plant in the Iranian population,while if the purpose of the production and cultivation of anguze is to use its antioxidant effects, the cultivation of the Afghanestanian population is more suitable.

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شناسایی ترکیبات شیمیایی تشکیل دهنده اسانس و بررسی اثرات آنتی‌اکسیدانی آنغوزه ایرانی و افغانستانی

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چکیده

در این مطالعه ترکیبات تشکیل دهنده اسانس، اثرات آنتی اکسیدانی و میزان فنل و فلاونوئید آنغوزه در دو جمعیت ایرانی و افغانستانی مورد بررسی قرار گرفته است. شناسایی ترکیب های تشکیل دهنده اسانس توسط دستگاه GC-MS صورت گرفت. بررسی اثرات آنتی اکسیدانی با استفاده از روش‌های ۲و۲ دی فنیل ۱–پیکریل هیدرازیل و بتاکاروتن – لینولئیک اسید انجام شد. محتوای ترکیبات فنلی و فلاونوئیدی نیز به ترتیب با استفاده از استانداردهای گالیک اسید و کوئرستین اندازه گیری شد. نتایج نشان داد که پروپنیل سک بوتیل دی‌سولفید (۴۲/۷۲٪)، استون دی متیل مرکاپتول(۱۴/۸۴٪) و گاما ادیسمول (۸.۶۰٪) فراوان ترین ترکیبات موجود در اسانس آنغوزه ایرانی است، پروپنیل سک بوتیل دی سولفید (۳۹/۶۱٪)، استون و دی متیل مرکاپتول (۱۸/۲۵٪)، و ۸ اتیل پنتادی دکان (۱۷/۹۷٪) فراوان ترین ترکیبات تشکیل دهنده اسانس آنغوزه افغانستانی بودند .میزان فعالیت آنتی اکسیدانی انغوزه ایرانی در روش DPPH قدرت بیشتری را نشان داد در حالی که در روش بتاکاروتن– لینولئیک اسید میزان فعالیت آنتی‌اکسیدانی نمونه افغانستانی بیشتر بود. محتوای ترکیبات فنلی و فلاونوئیدی در جمعیت افغانستانی بیشتر از جمعیت ایرانی بود. در مجموع نتایج نشان داد که دو جمعیت مورد بررسی در این پژوهش از نظر نوع و درصد ترکیبات تشکیل دهنده اسانس، قدرت آنتی اکسیدانی و محتوای فنل و فلاونوئید دارای تفاوت هایی با هم هستند. این تفاوت‌ها احتمالاً ناشی از تفاوت در عوامل اکولوژیکی مانند زیستگاه های محل رویش این جمعیت ها و تاثیر این عوامل برفاکتورهای مطالعه شده است.

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