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Chemical Profile of *Ferula Gummosa* Growing in the Saluk Protected Area of Iran and Comparing the Results with Other Habitats

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ABSTRACT

In this research, the volatile compounds of Oleo gum resin of *Ferula gummosa* were extracted from the protected area of Saluk located in North Khorasan province of Iran by water distillation method and the chemical compounds were identified by GC-MS and reported for the first time. In the studied plant essential oil, 42 compounds that made up 95.78% of essential oils have been identified, the main components of which are *β*-pinene (44.51%), α-pinene (11.49%), para-cymene (2.48%), 3-carene (5.74%), and 2-isopropyl 4-methylanisole (4.58%). In the following, five identified main compounds of this plant were compared with the main compounds of 26 ecotypes that were previously reported. By comparing the five main compounds of these 27 ecotypes, it was found that the main compounds of these samples are classified in the category of monoterpenes, sesquiterpenes, and esters, and also by drawing a cluster dendrogram using R software, the proximity and distance of these samples in terms of the type of main compounds, it is clustered into three clusters including samples with similar compounds. This study can be effective in continuing the research on eco-phytochemistry and ethnobotanical characteristics of *Frula gummosa*.

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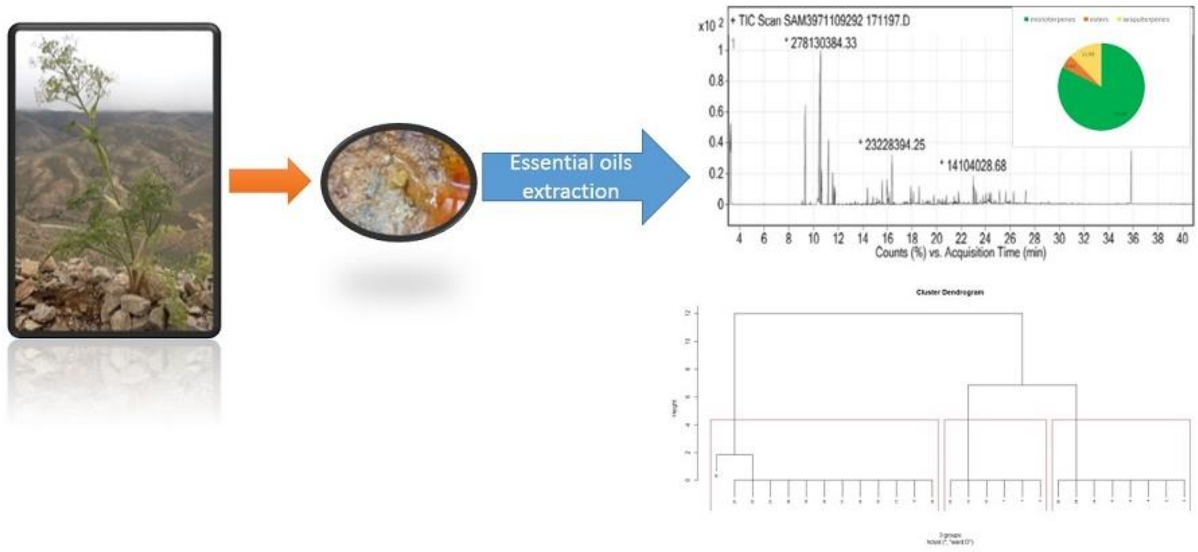
GRAPHICAL ABSTRACT



1. INTRODUCTION

Ferula gummosa is considered as one of the valuable medicinal plants and belongs to the Apiaceae (Umbelliferaea) family. This plant has 180 species, 15 of which are endemic to Iran, and known as galbanum, is distributed in the highlands of Iran, Afghanistan, and Central Asia [1]. Different species of this plant have been identified in Italy, Turkey, Tunisia, Algeria, Pakistan, Saudi Arabia, and India [2]. *Ferula gummosa* is a two-year herbaceous plant, which has mid-empty stems, and the alternate leaves of this plant are divided [1]. Of course, it is better to know that *Ferula gummosa* is one of the native plants of Iran and of course, this product has a juice that has a delicious smell and, of course, has countless properties. *Ferula gummosa* is one of the perennial plants that consists of thick stems and of course, the height of its stems reaches 3 meters with dissected leaves, showy yellow compound umbels and schizocarp fruits, growing wild in Iran and popularly referred to as “Baridje”. The leaves of this plant are grayish green. This plant bears fruit, and its fruit is oval and has a narrow side. This species produce a sort of milky latex or translucent oleo-gum-resin, turning to yellowish-brown as it is exposed to air. This exudate is made up of three fractions: (i) volatile oil (10–17%, w/w dw), (ii) gum (25%), and (iii) resin (40–64%) [3].Of course, the *Ferula gummosa* has different species and grows in the mountainous regions of Iran.

Ferula gummosa shows various therapeutic effects such as antibacterial [4], anti-edema, and dehydrating properties, disinfectant, milk booster, expectorant, stomach tonic, uterine tonic, and wound healer, and also as an antispasmodic, anti-bloating agent in traditional medicine [5].

The study of the anticonvulsant and toxic effects of *Ferula gummosa* essential oil has been done. These effects are related to the presence of compounds pinene and α-thujene in the essential oils [6,7]. *α*- and *β*-Pinene, a bicyclic monoterpenes, are the most widely distributed terpenoids in nature. These two compounds exhibit diverse biological activities, such as fungicidal agents, fragrances, flavors, and antiviral and antimicrobial agents [8].

Ferula gummosa essential oil–chitosan nanocomposite and the antibacterial activity of the prepared nanocomposite have been investigated against bacterial strains [9].

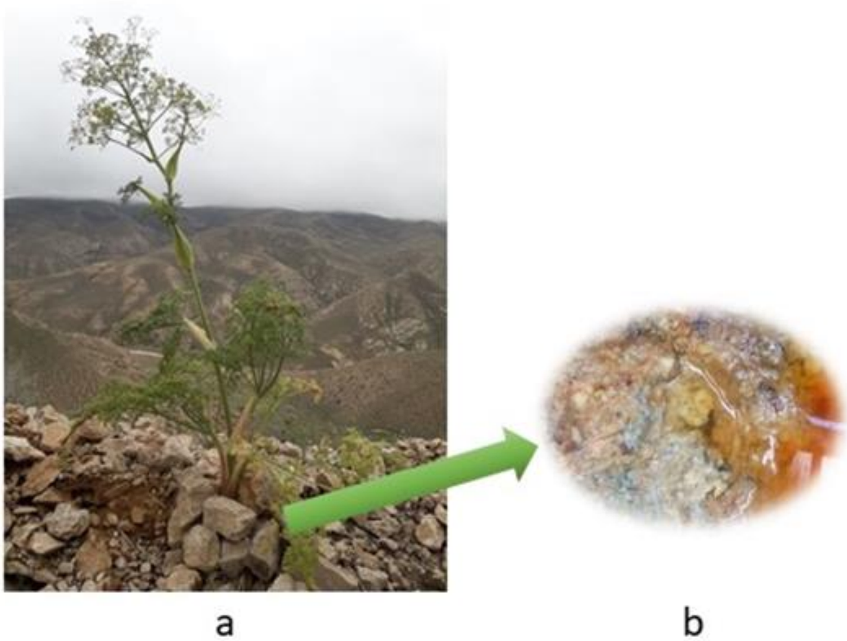


Fig 1. (a) *Ferula gummosa* and (b) Oleo gum resin of *Ferula gummosa*

In addition, α- and β-pinene are components of kidney and liver drugs [10]. They are also used as antimicrobial agents due to their toxic effects on membranes [11]. In addition, *α*- and *β*- pinene have inhibitory effects on breast cancer and leukemia [12]. The aim of this study is to identify the essential compounds of the resin of the *Ferula gummosa* grown in the protected Saluk Park for the first time, and also to compare its main compounds with the main compounds of 26 ecotypes reported in different articles. Determining the common main compounds in all ecotypes is one of the other goals of this research.

2. EXPERIMENTAL

2.1. Plant material

Oleogum resin *of Ferula gummosa* was collected from Saluk National Park, Northern Khorasan Province, in June 2022 (Fig 1). The voucher specimen (No. 563) was deposited in the herbarium of the Shaheed Beheshti University of Medical Sciences, Tehran.

2.2. Essential oils

The essential oils were obtained from the Oleo gum resin of *Ferula gummosa* for 3 hours using a Clevenger apparatus according to the Polish Pharmacopeia VII [13]. The method of extraction by distillation with water is used as a basic and common method in extracting essential oils from plants due to its simplicity, availability, and cheapness of the solvent (water) [14]. The essential oils were dried over anhydrous sodium sulfate and stored at 4 °C to identify compounds.

2.3. Analysis

GC analysis Analytical gas chromatography was carried out on a Shimadzu 15 A gas chromatograph equipped with split/split less injector (250 °C) and a flame ionization detector (250 °C). Chromatographic grade N_2 with a flow-rate of 1 mL/min was used as the carrier gas. The capillary column used was DB-5 (50 m × 0.2 mm; film thickness 0.32 μm).

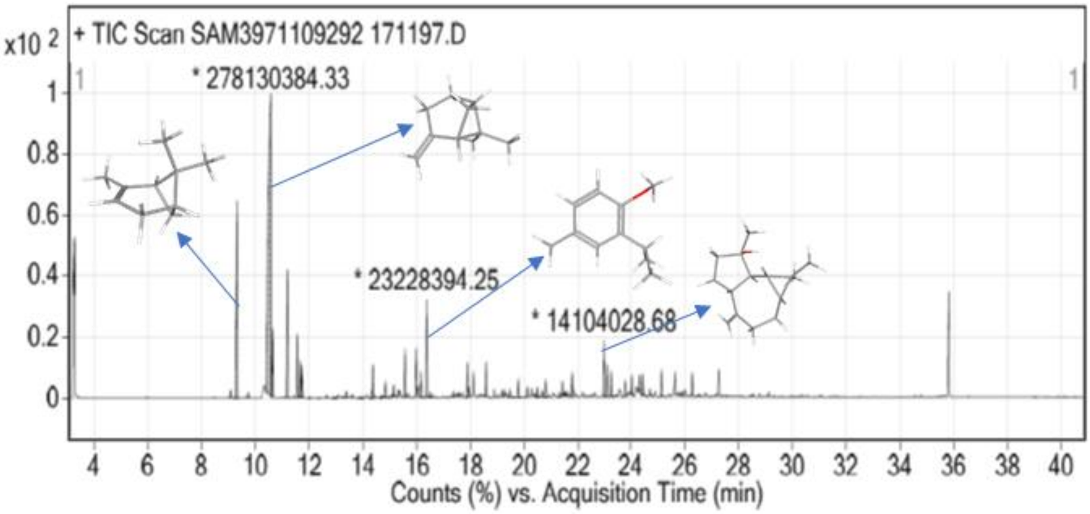


Fig 2. GC-MS chromatogram of the essential oil compounds of *Ferula gummosa*

The column temperature was kept at 60 °C for 3 min, and then it was heated to 220 °C at a rate of 5 °C/min, after which the temperature was kept constant at 230°C for 5 min.

GC-MS analysis A Hewlett-Packard 6890/5973 apparatus fitted with a fused silica HP-5MS (%5 Phenyl Methyl Siloxane) column (30 m × 0.25 mm; film thickness 0.25 µm) was used (Fig 2). The column temperature was kept at 50 °C for 3 min, programmed to 280 °C at a rate of 7 °C/min and kept constant at this temperature for 5 min. Chromatographic grade helium was utilized as the carrier gas (1 mL/min). All the mass spectra were recorded at 70 eV over the range m/z 50-500 amu. The interface temperature was set at 230 °C. Other instrumental parameters were as follows: trap temperature, 180 °C; emission current, 10 µA; scan time, 0.39 s; automatic gain control, 25,000; manifold and transfer line temperatures, 50 °C and 240 °C, respectively. In addition, hexane solutions of a homologous series of saturated paraffins (C_8-C_{25}) and the essential oils were analyzed by GC-FID and GC-MS, using both columns under the same conditions. The linear retention indices were then calculated for all volatile components. All chromatographic measurements were carried out in triplicate, and the mean of the retention times together with percentage composition of each component were taken into consideration. Duplicate times were discarded if they differed by more than 1 s, and the experiments were repeated again in duplicate. Identification and determination of the constituents of each oil were tentatively made by comparison of their mass spectral fragmentation patterns and retention indices (RI) relative to C_8-C_{25} n-alkanes both with those given in the literature [15] and those stored in a MS library (Wiley 275).

3. RESULTS AND DISCUSSION

3.1. GC-MS analysis

Hydrodistillation of the Oleo gum resin of *Frula Gummosa* yielded a clear oil in a yield of 0.40% (w/w) based on weight of the Oleo gum resin. The percentages and Kovats index of the recognized compounds are presented in Table 1. A total of 42 compounds were obtained representing 95.78 % of the Oleo gum resin oil. The main compounds found in the essential oils of Oleo gum resin of *Frula Gummosa* were β-Pinene(44.51%), α-pinene (11.49%), 3-carene (5.74%), 2-isopropyl-4-methylanisol (4.58%), and p-Cymene (2.48%) (Fig 2). The compounds found involved monoterpenes (74.59%), sesquiterpenes (11.33%), and esters (4.54%) of the total profile (Fig 3).

Table1. Chemical profiles of the essential oils of *Ferula gummosa* from Saluk National Park, Northern Khorassan Province

No.	**Component**[a]	**RI HP-5MS**[b]	**Area %**
1	α-Thujene (**MO**)	931	0.36
2	α-Pinene(**MO**)	940	**11.49**
3	Camphene(**MO**)	953	0.22
4	β-Pinene(**MO**)	979	**44.51**
5	Myrcene(**MO**)	990	2.47
6	3-Carene(**MO**)	1003	**5.74**
7	*p*-Cymene(**MO**)	1022	**2.48**
8	Limonene(**MO**)	1030	1.34
9	(E)-β-Ocimene(**MO**)	1048	1.21
10	γ-Terpinene(**MO**)	1057	0.02
11	*p*-Menthol(**MO**)	1065	0.15
12	Linanool(**MO**)	1097	0.06
13	Solusterol(**MO**)	1102	0.27
14	Fenchol(**MO**)	1117	0.02
15	Trans-Pinocarveol(**MO**)	1140	1.66
16	6,6-dimethyl-2-methylidenebicyclo[3.2.0]heptan-3-ol (**Other**)	1150	0.08
17	β-Pinene oxide(**MO**)	1158	0.06
18	Pinocarvone(**MO**)	1164	0.59
19	Isoamyl senecioate(**Ester**)	1180	0.44
20	3-Methylbut-3-enyl (E)-2-methylbut-2-enoate (**Ester)**	1190	0.25
21	Myrtenal (**MO**)	1195	1.94

22	Iso-Bornyl acetate(**Ester**)	1280	1.75
23	2-isopropyl-4-methyl anisole (**Other**)	1285	**4.58**
24	3-Methylbut-2-enoic acid, 4-cyanophenyl ester (**Ester**)	1287	0.30
25	Bornyl acetate(**Ester**)	1293	0.18
26	Pinocarvyl acetate(**Ester**)	1305	0.16
27	α-Terpineol acetate (**Ester**)	1347	1.24
28	α-Copaene (**Sq**)	1375	0.22
29	β-Elemene (**Sq**)	1389	0.57
30	2-tert-butyl-1,4-dimethoxybenzene (**Other**)	1405	0.66
31	α-Cedrene (**Sq**)	1412	0.50
32	Alloaromadendrene (**Sq**)	1450	0.45
33	Linalyl iso-valerate (**Ester**)	1461	0.22
34	α-Himachalene (**Sq**)	1472	0.22
35	β-Eudesmene (**Sq**)	1477	0.53
36	4-epi-cubebol (**Sq**)	1492	1.35
37	Spathulenol (**Sq**)	1576	2.26
38	Guaiol (**Sq**)	1597	1.25
39	Humulane-1,6-dien-3-ol (**Sq**)	1617	0.91
40	γ-Eudesmol (**Sq**)	1628	1.25
41	α-Cadinol (**Sq**)	1658	0.74
42	Bulnesol (**Sq**)	1665	1.08
	Total		95.78

[a] *Order of compounds is according to their elution from HP-5MS (30 m × 0.25 mm, 0.25 µm) column.*
[b]*Linear retention index calculated using a mixture of n-alkanes (C_8–C_{25}) respect to HP-5MS column.*
MO*: Monoterpenes,*
Sq*: Sesquiterpenes.*

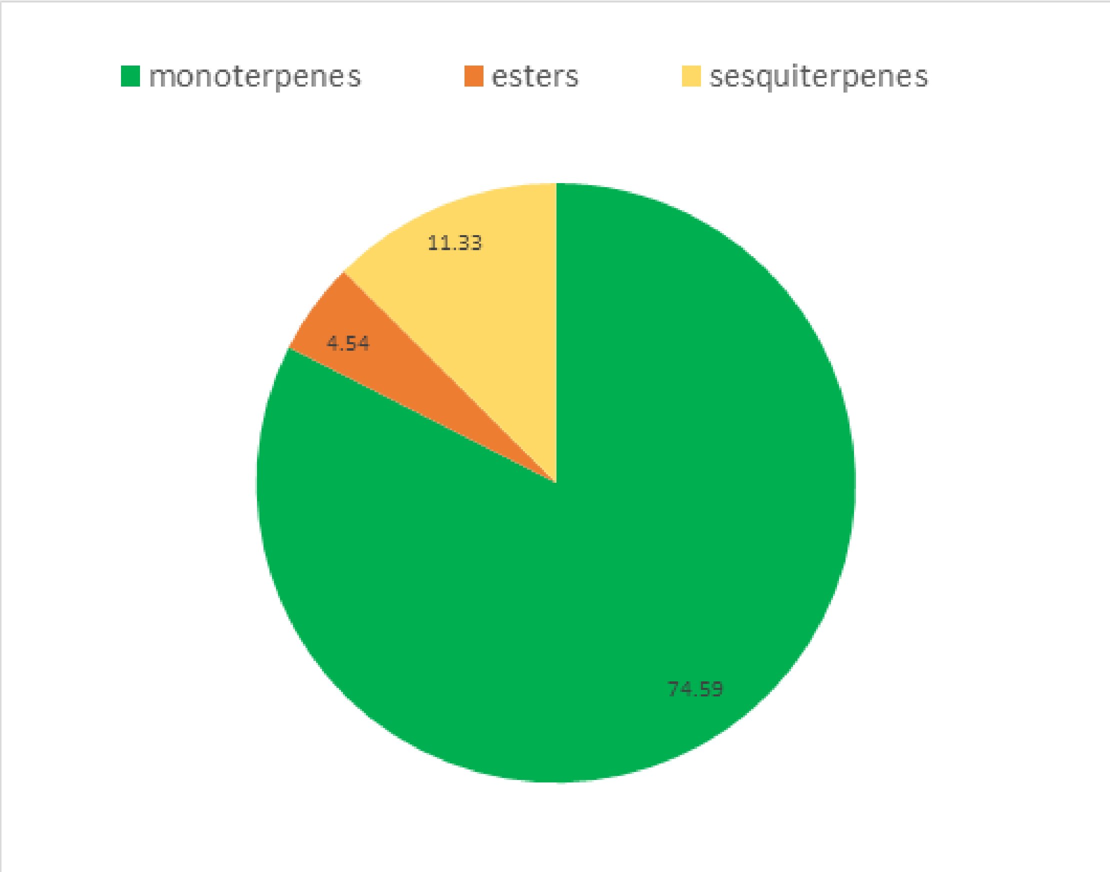


Fig 3. The percentage of types of compounds in the essential oils of Oleo gum *resin of Ferula gummosa*

3.2. Comparison of the main compounds of the studied plant essential oil with previous reports

In this research, the main essential compounds of Oleo gum resin *Ferula gummosa* collected from Saluk protected area in North Khorasan Province were compared with other studies conducted on the essential compounds of this plant in different regions. Among the 5 main compounds compared for each of the reports, almost β-pinene and α-pinene compounds have the highest composition percentage, and in general, in most reports, these compounds are present in larger amounts than other compounds.

Only in the case of reports FG_6, FG_{15}, FG_{20}, FG_{22}, and FG_{26}, α-pinene compound, and in the reported cases FG_{25}, FG_{24}, and FG_{21}, *β*-pinene and *α*-pinene compounds do not account for the highest percentage of transfused compounds (Table 2).

In this research, an attempt has been made to determine the relationship between the main compounds identified in the essential oils of this plant by studying various reports about the essential oils compounds in different locations. According to Table 2, it can be seen that in a number of reports, the main compounds in the essential oil of *Ferula gummosa*, it is completely the same and even the composition of their percentages is also the same (FG_2, FG_3, and FG_4).

Table2. Comparison of the main essential oil components isolated from *Ferula gummosa* species in the present study with literature data

Sample	Location	Main compounds	The technique of extracting essential oils	Ref.
FG_1	Bojnord- North Khorasan- Saluk protected area	*β*-pinene (44.51%), *α*-pinene (11.49%), *p*-Cymene (2.48%), 3-carene (5.74%), and 2-isopropyl-4-methylanisol (4.58%)	Hydrodistillation-Clevenger	Present Study
FG_2	Ploor(30 km northest of Tehran)	*β*-pinene (50.1%), *α*-pinene (18.3%), 3-carene (6.7%), α-thujene (3.3%), and sabinene (3.1%)	Hydrodistillation-Clevenger	6
FG_3	Lar region 120 km east of Tehran	*β*-pinene (50.1%), *α*-pinene (18.3%), 3-carene (6.7%), α-thujene (3.3%), and sabinene (3.1%)	Hydrodistillation-Clevenger	16
FG_4	Esfahan-khorasgan	*β*-pinene (50.1%), *α*-pinene(18.3%), 3-carene (6.7%), *α*-thujene (3.3%), and sabinene (3.1%)	Hydrodistillation-Clevenger	17
FG_5	Fereydunshahr	*β*-pinene (30.68%), *α*-pinene (27.97%), Limonene (6.78%), α-thujene (5.61%), and Fenchyl acetate (6.7%)	Hydrodistillation-Clevenger	18
FG_6	Kashan	*β*-pinene (28.44%), 3-carene(8.86%), Limonene (5.09%), Germacrene -D (4.45%), and Camphene (5.04%)	Hydrodistillation-Clevenger	18
FG_7	Semirom	*β*-pinene (34.75%), *α*-pinene (31.40%), Limonene(5.89%), Fenchyl acetate (5.73%), and α-phellandrene (4.23%)	Hydrodistillation-Clevenger	18
FG_8	Ploor	*β*-pinene (26.85%), *α*-pinene(19.13%), 3-carene(7.29%), methylthiobenzamide (5.50%), and *α*-phellandrene (7.89%)	Hydrodistillation-Clevenger	18
FG_9	Emam pahnak-Lar	*β*-pinene (51.55%), *α*-pinene(19.69%), 3-carene (7.57%), *α*-thujene (4.39%), and Limonene (1.87%)	Hydrodistillation-Clevenger	18
FG_{10}	Nemadkovsar 1-Lar	*β*-pinene (50.12%), *α*-pinene(17.54%), 3-carene (6.23%), α-Gurjunene (5.61%), and Limonene (4.84%)	Hydrodistillation-Clevenger	18
FG_{11}	Garmsar	*β*-pinene (32.59%), *α*-pinene(31.04%), Cyclofenchene (1.56%), Valencene (5.38%), and	Hydrodistillation-Clevenger	18

		Limonene (3.32%)		
FG_{12}	Tehran	*β*-pinene (31.63%), *α*-pinene(33%), Fenchyl acetate(3.67%), and α-phellandrene(6.81%), and Limonene (2.83%)	Hydrodistillation-Clevenger	18
FG_{13}	Ilam	*β*-pinene (33.3%), *α*-pinene(33.91%), Fenchyl acetate(4.99%), Germacrene B(5.92%), and Limonene (5.37%)	Hydrodistillation-Clevenger	18
FG_{14}	Nemadkovsar 2-Lar	*β*-pinene (49.79%), *α*-pinene(16.65%), 3-carene (6.07%), *α*-thujene (2.73%), and Limonene (5.24%)	Hydrodistillation-Clevenger	18
FG_{15}	Semnan	*β*-pinene (40.99%), 3-carene (11.80%), Limonene (9.15%), Nerolidol (5.80%) and *β*-phellandrene (6.52%)	Hydrodistillation-Clevenger	18
FG_{16}	Mashhad	*β*-pinene (37.91%), α-pinene(9.78%), 3-carene (9.10%), Calarene (6.33%), and α-Patchoulene (5%)	Hydrodistillation-Clevenger	18
FG_{17}	Roshehvamasly-Firouzkouh	*β*-pinene (69.15%), *α*-pinene (4.83%),trans-Caryophyllenen(2.52%), Calarene (2.73%), and γ-Gurjunene (5.14%)	Hydrodistillation-Clevenger	18
FG_{18}	Reef-Firouzkouh	*β*-pinene (59.21%), *α*-pinene (5.01%), Guaiol (4.33%), *γ*-Selinene (8.40%), and Valencene (4.60%)	Hydrodistillation-Clevenger	18
FG_{19}	Sarake-Firouzkouh	*β*-pinene (67.19%), *α*-pinene(4.26%), Calarene (5.05%), Valencene (3.42%), and Nerolidol (4.24%)	Hydrodistillation-Clevenger	18
FG_{20}	Natanz	*β*-pinene (34.54%), Camphene(14.09%),% Calarene(6.71%), *α*-Patchoulene(7.82%), and *α*-Gurjunene (15.02%)	Hydrodistillation-Clevenger	18
FG_{21}	Firuzkooh	Myrtenol (3.76%),Guaiol (3.42%), α-Eudesmol (4.30%), Bulnesol (7.11%), and α-Bisabolol (3.55%)	Steam distillation	19
FG_{22}	Lorestan	*β*-pinene (21.79%), 3-carene (8.22%), Trans- propenyl sec-butyl disulfide (5.603%), *p*-Menth-2-en-1-ol (15.35%), and *α*-maaliene (5.156%)	Hydrodistillation-Clevenger	20

FG_{23}	Firouzkooh	*β*-pinene (51.83%), *α*-pinene(6.44%), 3-Carene (5.47%), *β*-Phellandrene (4.16%), and Carvacrol methyl ether (4.06%)	Hydrodistillation-Clevenger	4
FG_{24}	Jajarm-north khorasan	γ-Elemene (14.1%), Epizonaren (6.2%), Viridiflorene (8.1%), γ-Bisabolene (10.7%), and Germacrene B (11.8%)	Hydrodistillation (HD)	2
FG_{25}		*β*-Elemene (5.1%), γ-Elemene (6.5%), Aromadendrene (17.6%), *γ*-Bisabolene (6.3%), and Germacrene B (16.2%)	Solvent-free microwave extraction (SFME)method	
FG_{26}	Kashan	*β*-pinene (58.8%), 3-carene (12.1%), α-pinene (5.7%), *β*-myrcene (4.6%), and limonene(4%).	Hydrodistillation (HD)	21
FG_{27}	North Khorasan, Esfarayen	*β*-pinene (40.7%), *α*-pinene (16.2%), *β*-Phellandrene (22.7%),σ-cadinene (7.2%), and *α*-muurolene (1.3%)	Hydrodistillation-Clevenger	22

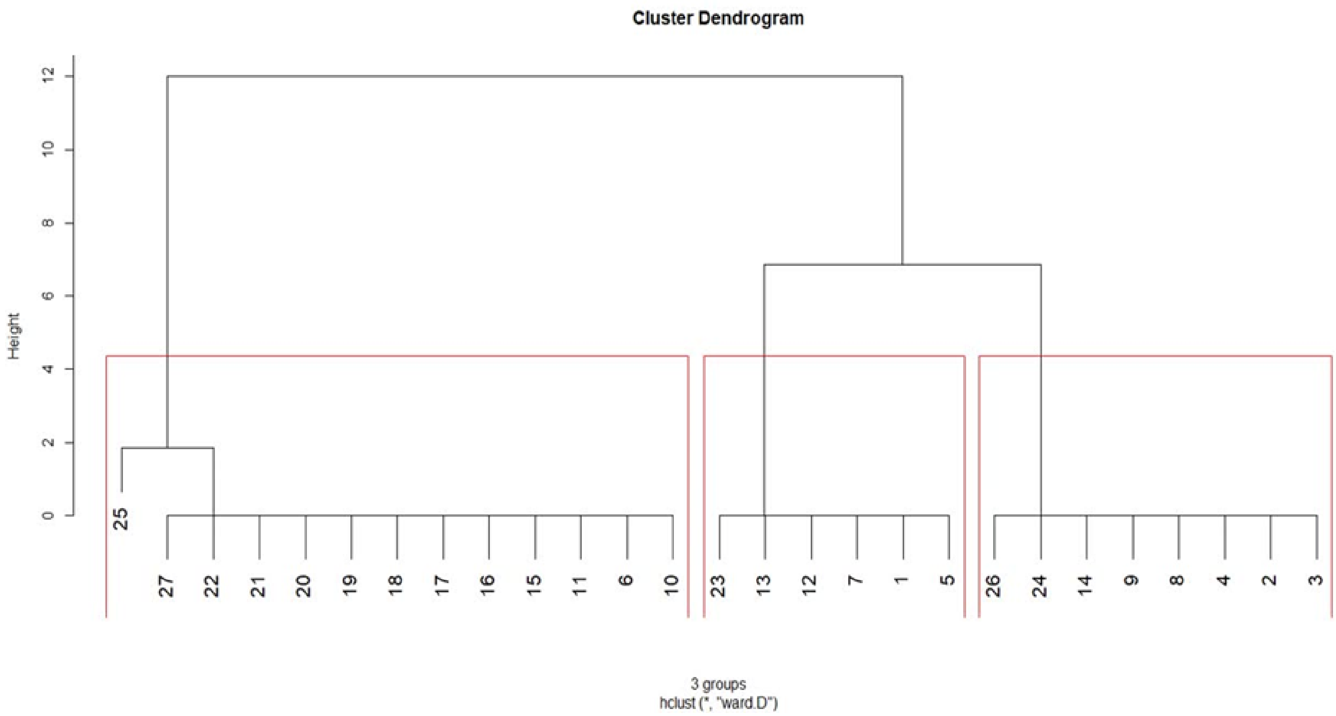


Fig 4. Dendrogram of twenty-seven ecotypes *Ferula gummosa* according to five main compounds of their essential oils using grouped into three distinctive clusters

3.3. Statistical analysis

To perform the analysis, the analytical data was done using R software version 3.6.1, with the Ward clustering method for the relationship and proximity of essential oil compounds of different samples (27 ecotypes) of *Ferula gummosa*.

The Euclidean distance clustering method was used to classify 27 ecotypes of *Ferula gummosa*. In this dendrogram, 27 ecotypes were screened based on the relative percentages of 3 categories of dominant compounds in the main essential oil compounds (monoterpenes, sesquiterpenes, and esters) (Table 3).

This clustering has been done according to the 5 main compositions of each report as well as the present study. As mentioned before, these compounds are classified into 3 categories of monoterpenes, sesquiterpenes, and esters. In the category of monoterpenes, sample FG_{26} with 85.2% is the most monoterpene, and regarding the category of sesquiterpenes, sample FG_{25} has the highest sesquiterpenes content with 51.7%. Also, sample FG_7 with 6.71% has the most esters in the esters category.

Table3. Classification of 5 main compounds of 27 ecotypes of *Ferula gummosa* in three main categories

Sample	Monoterpenes	Sesquiterpenes	Esters
FG_1	64.21	-	4.58
FG_2	81.5	-	-
FG_3	81.5	-	-
FG_4	81.5	-	-
FG_5	77.74	-	**6.71**
FG_6	47.43	4.45	-
FG_7	85	-	5.73
FG_8	61.16	-	-
FG_9	85.07	-	-
FG_{10}	78.73	5.61	-
FG_{11}	68.51	5.38	-
FG_{12}	77.94	-	3.67
FG_{13}	77.56	-	4.99
FG_{14}	80.48	-	-
FG_{15}	68.46	5.8	-
FG_{16}	56.79	11.33	-
FG_{17}	73.98	10.39	
FG_{18}	64.22	17.33	-
FG_{19}	71.45	12.71	
FG_{20}	78.63	29.55	
FG_{21}	3.76	18.38	-
FG_{22}	45.36	5.15	-
FG_{23}	71.96	-	4.06
FG_{24}	44.7	-	-
FG_{25}	-	**51.7**	-
FG_{26}	**85.2**	-	-
FG_{27}	79.6	8.5	-

4. CONCLUSION

The present study is to determine the essential oils compound of *Ferula gummosa* collected from Saluk protected area in North Khorasan province and compare the five main components of this sample with other samples reported in the articles between2001-2020. Retention Indices was identified using GC-MS spectrometry information as well as the reliable sites of NIST and Pherobase and reported in Table 1. Among the 42 identified components of the studied sample were *β*-pinene (44.51%), α-pinene (11.49%), 3-carene (5.74%), 2-isopropyl-4-methylanisol (4.58%), and *p*-Cymene (2.48%). For comparison, 5 main compounds of each sample were extracted from the reported article and included in Table2. Given that the majority of the components of the samples are monoterpene compounds and among them α- and β-pinene are the main compounds, the investigation and introduction of this compound can be effective in understanding the importance of these compounds. One of the most abundant terpenes in nature is pinene [23].

In nature, this compound has been isolated and known in two structural isomers, α and β [24]. α- and β-pinene are the main components of resin compounds, especially in rosemary and lavender plants [25]. *α*- and *β*-pinene are used in the perfumery industry [26]. In terms of medicinal effects, *α*- and *β*-pinene present in several medicinal plants in traditional medicine are used to treat various diseases such as digestive disorders, colds and fevers, rheumatism, high blood pressure, seizures, snake bites, inflammation and pain, cancer ,fungal infection, etc. are used [27-30]. According to the dendrogram (Fig 4), the clustering is based on the type of main compounds present in these 27 ecotypes, which are located in the first cluster of (FG_2, FG_3, FG_4, FG_8, FG_9, FG_{14}, FG_{24}, and FG_{26}) samples. In this cluster, according to Table 3, all the samples in this cluster contain monoterpene compounds, and this factor causes the similarity of these samples, and they are placed in the same cluster in the clustering dendrogram, as mentioned, the compounds of samples FG_2, FG_3, and FG_4 have been reported the same and this has made these compounds to be more similar and to be placed in the same cluster. The second cluster contains examples of (FG_1, FG_5, FG_7, FG_{12}, FG_{13}, and FG_{23}). It can be seen carefully in the clustering dendrogram as well as in Table 3 that the samples are placed in this cluster whose main compounds are in the group of monoterpenes and esters and these samples do not contain sesquiterpene compounds in their main essential oil compounds. In the same vein, the third cluster includes the samples whose monoterpene and sesquiterpene compounds contain the highest percentage of their essential oils. Samples (FG_6, FG_{10}, FG_{11}, FG_{15}, FG_{16}, FG_{17}, FG_{18}, FG_{19}, FG_{20}, FG_{21}, FG_{22}, FG_{25}, and FG_{27}) are included in this cluster. With a little precision, it can be seen that sample FG_{25} is placed in this cluster with a little difference and distance compared to other samples, and by looking at Table 3, it is clear that sesquiterpene compounds have the highest percentage composition in the main compounds of this sample and monoterpene compounds. The samples are not important, so this sample is placed in this cluster with a little distance from the rest of the group due to this similarity (sesquiterpenes) and difference (absence of monoterpenes).

According to the identified compounds of Oleo gum resin of *Frula gummosa* from Saluk protected area for the first time, the highest percentage of compounds are monoterpenes (74.59%), and among these compounds, α- and *β*- pinene have the highest percentage among compounds. Finally, in this article, another sample of *Frula gummosa* from a different region, which has not been reported so far, was studied and its essential components were isolated and identified. In this case, as in the previous 26 reports, the most α- and β-pinene are the main components of the essential oil, and it is suggested that *Frula gummosa* be introduced as a good and rich source of pinene compounds for the treatment of the mentioned diseases, and this comparison can be a guide to choose the desired sample from the climate that is the most pinene compounds have been reported from it. Carrying out this comparison can be a strategy for ecophytochemical studies. Also, in future studies, according to the availability of information and physical and chemical indicators such as altitude, soil chemistry, etc. it is possible to study the effects of these factors on the percentage and composition of essential oils.

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Ethical Approval

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Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

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