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Comparative study of volatile oils extracted from aerial parts of *Ferula gummosa* using carbon nanotube profile and hydrodistillation and antioxidant activities of methanolic extract

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Abstract
Ferula gummosa, a native medicinal plant growing in Iran, was studied in this literature. Two different extraction methods were used for a comparative study of Volatile Oils Extracted from aerial parts of *Ferula gummosa*: Hydrodistillation (HD) and Carbon Nanotube Profile. Volatile oils analyzed by GC and GC-MS presented 32 and 34 components constituting 85.792% and 84.903% of the total oils, respectively. Carbon Nanotube Profile were richer in oxygenated sesquiterpenes. Their major compounds were such as β-moaliene (19.431%), β-Gurjunene (10.23%), Allo-aromadendrene (7. *et al*), Aromandrene (6.528%). The antioxidant activities were studied with the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging method. Generally, methanolic extract showed high antioxidant activities. Comparison between hydrodistillation and Carbon Nanotube Profile presented numerous distinctions. Several advantages with Carbon Nanotube Profile were observed, This method is a new, fast and easy.

Keywords: *Ferula gummosa,* volatile oils, carbon nanotube profile, hydrodistillation, antioxidant activity

1. Introduction
Ferula gummosa Boiss. (Apiaceae) is perennial plant native to central Asia, growing in the northern and western parts of Iran and blooms once in its several years' life span [1]. Nomads of southwest Iran call this plant 'Barijeh' and traditionally use its resin for the treatment of diarrhea. They eat a small piece of the resin and believe it to be a very effective anti-diarrheal herbal medicine [2]. In Iranian ancient medicine, the gum obtained from the aerial parts of this plant has been used for stomach pain, chorea, epilepsy and as a wound-healing remedy [3]. In recent years there are some reports regarding the main effects of this plant. An anti-nociceptive activity has been shown for the hydro alcoholic extract of aerial parts [4] and acetone extract of *F. gummosa* seed and root has been reported previously [5]. Furthermore, a methanol-chloroform (1:1) extract of *F. gummosa* and its fractions have alleviated the morphine withdrawal syndrome induced by naloxone [6]. The anticonvulsant potential of an essential oil 10 and the antibacterial activity of the seed [7] and anti-inflammatory activity of the seed and root of *F. gummosa*13 have been reported previously. The composition of the essential oil of the fruit of the plant has been determined and it has been shown that terpenoid compounds such as alpha-pinene, betapinene, 3-carene, alpha-thujene and sabinene are abundant in this plant [8].

2. Experimental
2. 1 Plant material
The plants *Ferula gummosa* Boiss.(Apiaceae) was identified and authenticated by Prof. Dr. Nasser Akbari at the Department of Agronomy, faculty of agriculture, University of Lorestan. The voucher specimens have been deposited at the Herbarium of the Lorestan University. The aerial parts of the plant were collected from Lorestan university campus area in May 2011 and dried at 30 °C for 4 days without applying any heat treatment to minimize the loss of active components. Dried materials were kept in deep freeze until use.

2.2 Isolation of volatile components

2.2.1 Hydrodistillation method

The sample (100 g of dried material was charged with a particle size of about 500 µm) was submitted to hydro-distillation for 2.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile distillate was collected over anhydrous sodium sulphate and after filtration, immediately injected to GC/MS. The yield of the oil was 2.05% v/w based on dry plant weight

2.2.2 Carbon Nanotube Profile method

Fresh powdered (10g) of plant were subjected to nanotube (MWNTs 20-40 nm length 5-15 micro meter S.S area 40-300 m2/g) in a petri dish –type apparatus heated for 4h. at 40C then washed by n-hexane. The yield (V/W) of volatile oil was 0.2%. The volatile oil was dried over anhydrous sodium sulfate and stored at 4 C for analysis

2.3 GC-MS analysis

GC/MS analysis of the oil was carried out on an Agilent HP-6890 gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent HP-5973 mass selective detector in the electron impact mode (ionization energy: 70eV), operating under the same conditions as described above, using a HP-5MS 5% phenylmethylsiloxane capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Restek, Bellefonte, PA). Retention indices were calculated for all components using a homologous series of n-alkanes injected in conditions equal to the sample 671 one. Identification of components of essential oil was based on retention indices (RI) relative to nalkanes and computer matching with the Wiley7n.L libraries, as well as comparisons of the fragmentation pattern of the mass spectra with data published in the literature [9] Some commercially available components of the essential oil were also co- injected for further confirmation of their identification.

2.4 Measurement of Antioxidant Activity

The antioxidant activities of methanolic extract from fresh aerial parts of Ferula was evaluated following the model described by Brand-Williams *et al*. with some changes. The absorbance measurements of various samples were performed using a Helios Omega spectrophotometer model (Thermo Fisher Scientific, St. Herblain, France). Maximum absorption of the radical DPPH was identified at 517 nm in methanol.

A solution of DPPH (0.2 mM) in methanol was prepared as well as a series of EO solutions in methanol from 20 to 5000 µg/mL from a stock solution of 10 mg/mL. The concentrations of BHT, quercetin and gallic acid used as references were located in the ranges between 2 µg/mL and 25 µg/mL, 1 µg/mL and 12 µg/mL, and 0.3 µg/mL and 5.0 µg/mL, respectively. They were made from stock solutions of 50 µg/mL, 30 µg/mL, and 10 µg/mL, for BHT, quercetin and gallic acid, respectively. A sample of 1.5 mL of DPPH was added to 2.5 mL of diluted methanolic extract. The mixture was vigorously stirred and left for 30 min at room temperature in a dark area, away from light sources. Measurements were carried out with a blank prepared by mixing 2.5 mL of the same essential oil extract with 1.5 mL of methanol. Tests were performed with three successive repetitions (chart1).

3. Results and Discussion

The volatile oil of barks of *Ferula gummosa* was extracted by hydrodistillation method and was analyzed by GC and GC-MS (Chromatogram 1). The chemical composition of the *Ferula gummosa* is presented in Table 1. A total of 32 compounds were identified, which constitute 85.792% of the volatile oil. β- Gurjunene (4.356%), p-ment- 2en- 9 ol E (15.356%), Beta-pinene (21.790%), Trans propenyl sec butyl disulfide (5.603%), identified as major components. On the other hand, The chemical composition of the *Ferula gummosa* extracted by Carbon Nanotube Profile is presented in Table 2 (Chromatogram 2). A total of 34 compounds were identified, which constitute 98% of the volatile oil. The volatile oil of *Ferula gummosa* contains β-moaliene (19.431%), β-Gurjunene (10.23%), Allo-aromadendrene (7.518%) and Aromandrene (6.528%) total compound identified is 84.903%.

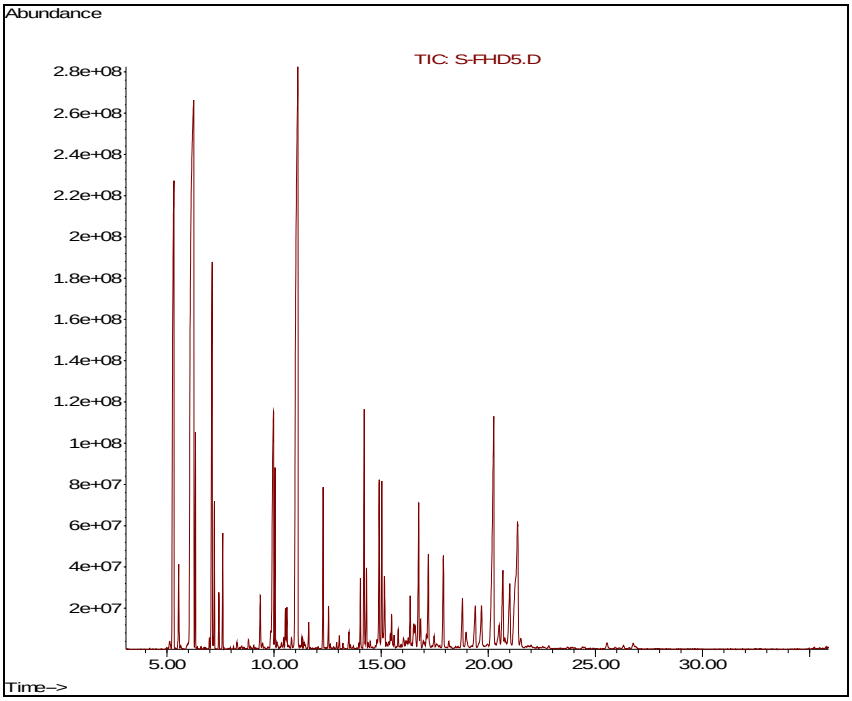

Table 1: Composition of essential oil from *Ferula gummosa* by hydrodistillation method

Row	Compound	KI	RI	Percentage
1	Camphene	954	5.31	0.714%
2	Beta- pinene	979	5.27	21.790%
3	Myrcene	991	5.54	1.482%
4	D- limonene	1029	6.11	3.986%
5	Delta- 3- careen	1031	6.32	8.217%
6	Allo-Ocimene	1050	7.1	0.129%
7	Gamma- terpinene	1060	7.2	0.908%
8	Trans- pinocarveol	1139	9.3	0.547%
9	Trans propenyl sec butyl disulfide	-	9.91	5.603%
10	Myrtenal	1196	10.52	0.346%
11	Myrtenol	1196	10.60	0.377%
12	p-ment- 2en- 9 ol E	1199	11.08	15.356%
13	Rubean	-	11.68	0.221%
14	Endobornyl acetate	-	12.20	1.134%
15	3- Caren- 4ol	-	12.55	0.339%
16	Alpha- Cabebene	1351	13.40	0.175%
17	α-copaene	1377	14.03	0.521%
18	Beta- elemene	1391	14.19	0.701%
19	α-Gurjunene	1410	14.30	0.829%
20	Trans- caryophyllene	1419	14.90	1.441%
21	α-Guaiene	1440	15.03	1.868%
22	Alloaromadendrene	1460	15.13	0.392%
23	β-selinene	1490	16.35	0.535%
24	α-Farnesene	1506	17.2	1.188%
25	D- cadinene	1523	17.89	1.534%
26	Calarene	-	18.96	0.791%
27	α-Eudesmol	1654	19.39	0.943%
28	β-moaliene	-	19.68	5.156%
29	Agarospirol	1648	20.24	1.358%
30	Aromandrene	-	20.67	1.329%
31	β-Gurjunene	-	21.08	4.356%
32	Aristolene	1763	21.36	1.526%
	Total		85.792	

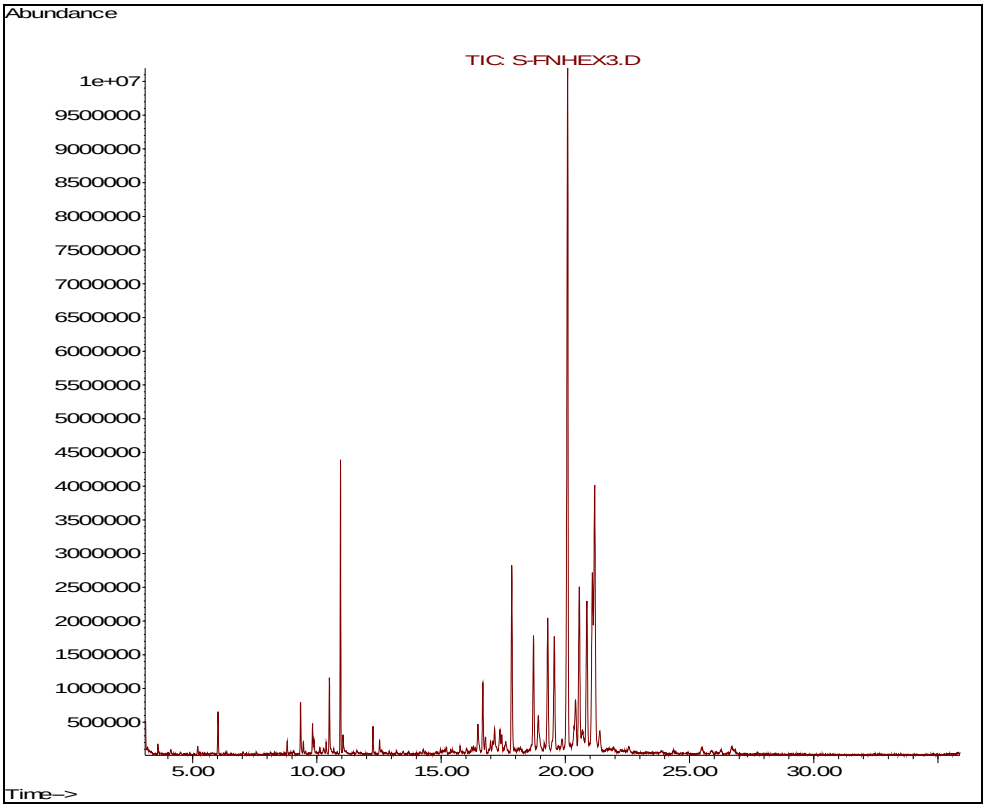

Table 2: composition of volatile oil from *Ferula gummosa* by nano tube

Row	Compound	KI	RI	Percentage
1	Camphene	954	5.13	0.021
2	Beta- pinene	979	5.27	0.837
3	Myrcene	991	5.54	0.105
4	D- limonene	1029	6.11	0.219
5	Delta- 3- careen	1031	6.32	0.060
6	Allo-Ocimene	1050	7.1	0.132
7	Gamma- terpinene	1060	7.2	0.186
8	Trans- pinocarveol	1139	9.3	1.114
9	Trans propenyl sec butyl disulfide	1167	9.91	0.891
10	Myrtenal	1196	10.52	1.485
11	Myrtenol	1196	10.60	0.375
12	p-ment- 2en- 9 ol E	1199	11.08	0.699
13	Rubean	1210	11.61	0.462
14	Endobornyl acetate	1285	12.20	0.726
15	3- Caren- 4ol	1315	12.55	0.642
16	Alpha- Cabebene	1351	13.40	0.564
17	α-copaene	1377	14.03	0.141
18	β-Cubebene	1388	14.19	2.298
19	Beta- elemene	1391	14.30	0.627
20	α-Gurjunene	1410	14.90	0.420
21	Trans- caryophyllene	1419	15.03	0.420
22	α-Guaiene	1440	15.13	0.393
23	Allo-aromadendrene	1460	16.35	7.518
24	α-Amorphene	1485	17.2	2.226
25	β-selinene	1490	17.89	0.558
26	α-Farnesene	1506	18.96	5.067
27	D- cadinene	1523	19.39	3.921
28	Calarene	1609	19.68	4.986
29	α-Eudesmol	1654	20.24	5.172
30	β-moaliene	1671	20.67	19.431
31	Agarospirol	1689	20.69	5.982
32	Aromandrene	1693	21.08	6.528
33	β-Gurjunene	1723	21.36	10.23
34	Aristolene	1763	21.39	0.471
	Total			84.903%

4. Conclusion

In this study, it was shown that the chemical composition of the volatile oil of aerial parts of Ferula depends on the method of extraction and has also differences compared to those reported in the literature, however with less impact in terms of yields. Many other studies show extreme variability due to geographical location and growth stage.

Carbon Nanotube Profile was found to be highly effective enabling a considerable reduction in extraction time, providing an essential oil with a chemical composition enriched in oxygenated compounds but with yields similar to those of steam distillations and also it consumed much less energy than hydrodistillation, in both stages of extraction and condensation. These benefits show that Carbon Nanotube Profile is a good alternative to other extraction techniques and it can thus be recommended for the extraction of natural volatiles (table3).

Methanolic extract of *Ferula gummosa* had a high antioxidant activity (chart1). However, this extract showed a great antioxidant activity in the DPPH• test. This can be explained by the presence of a greater number of phenolic compounds and oxygenated molecules.

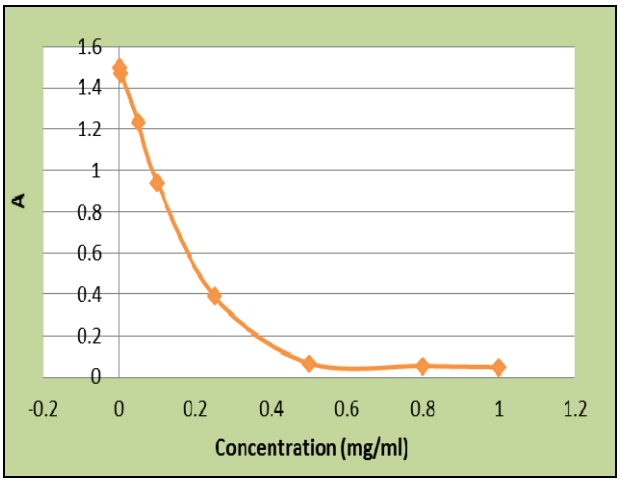

Chart 1: the chart of DPPH test According to Concentration and absorption

Table 3: Compare type of extracted terpenes with two methods

method CNT (W/W %)	method HD (W/W %)	type of terpenes
1.560	37.826	hydrocarbon monoterpene
5.307	18.099	oxygenated monoterpene
37.758	12.824	hydrocarbon sesquiterpene
11.064	2.301	oxygenated sesquiterpene
29.661	10.312	Unknown Compounds
14.650	18.620	other Compounds

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