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DETERMINATION OF BIOACTIVE AND FRAGRANT MOLECULES FROM LEAVES AND FRUITS OF *Ferula assa-foetida L.* GROWING IN CENTRAL IRAN BY NANO SCALE INJECTION

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The essential oils of leaves and fruits of *Ferula assa-foetida* L., which belongs to the Umbelliferae family and grows in central Iran, were obtained by hydrodistileation method and analyzed by GC and GC-MS apparatus. The amount of the samples injected were 1.0 nL (diluted 1.0 µL of sample in 1000 ml of n-pentane, v/v), Twenty three and Twenty five bioactive, flavour and fragrance molecules, including 86.85% and 91.13% of the total components detected, were identified in this plant, which Trans-2-undecen-1-ol (17.26%) and γ-elemene (32.21%) were major components in leaves and fruits oils, respectively.

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1. Introduction

Plants have been a constant source of drugs and recently, much emphasis has been placed on finding novel therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs [1]. *Ferula* is a genus of about 130 species of flowering plants in the family Umbelliferae, native to the Mediterranean region and central Asia [2, 3]. The Iranian flora comprises of 30 species of *Ferula* genus, which some are endemic [4].

Several species of this genus have been used in folk medicines [5], and investigations on the ferula species have indicated antinociceptive, anti-inflammatory and antipyretic effects [6], contraceptive action [7, 8] and smooth muscle relaxant activity [9]. This genus is well documented as a good source of biologically active compounds such as sesquiterpene coumarins [10] and sesquiterpene [11]. Also, the *Ferula* genus has been found to be a rich source of gum-resin [2].

Asafoetida (*Ferula assa-foetida)*, is a species of *Ferula* grows in Iran, Afghanistan and Kashmir. It has an unpleasant smell, is herbaceous and perennial and grows up to 2m height [12, 13], with stout, hollow, somewhat succulent stems 5-8 cm diameter at the base of the plant. The leaves are 30-40 cm long, tripinnate or even more finely divided, whit a stout basal sheath clasping the stem. The flowers are yellow, produced in large compound umbels [14]. *Ferula assa-foetida* has several medicinal properties including antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, nervine, analgesic, anthelminitic, aphrodisiac and antiseptic [15].

Iranian *Ferula assa-foetida* contains gum extract (glucose, galactose, L-arabinose, rhamnose and glucuronic acid etc.), resin (ferulic acid esters, free ferulic acid, coumarin derivatives) and volatile oils (sulphur-containing compounds and various monoterpenes) [16].

Literature survey revealed that the essential oils of the leaves and fruits of this plant in central Iran have not been chemically studied previously. The present paper deals with the detailed analysis of two oils by capillary GC and GC-MS with the determination of the percentage bioactive and fragrant molecules by nano scale injection.

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2. Material and methods

Plant Material

Leaves and fruits of *F. assa-foetida* were collected in June 2008 in Kashan area (province of Isfahan, Iran) at an altitude of 2850 m. Leaves and fruits were dried in the shade (at room temperature). The voucher specimens of the plant were deposited in the herbarium (Voucher NO.KBGH 5237) of Research Institute of Forests and Rangelands, Kashan, Iran.

Isolation of the Essential Oils:

The air-dried and ground leaves (70g) and fruits (200g) of *F. assa-foetida* were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus [32]. After decanting and drying over anhydrous sodium sulfate, the corresponding dark yellow oils were recovered from the leaves and fruits in a yields of 0.043%, 0.49% (w/w), respectively and stored at low temperature (4ºC) further analysis.

Gas Chromatography (GC):

GC analyses of two oils were performed on an Agilent HP-6890 gas chromatograph equipped with flame ionization detector (FID) and an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness, 250 nm). The oven temperature was programmed as follows: 50ºC (3 min), 50-130ºC (3ºC/min), 130ºC (2 min), 130-200ºC (2ºC/min), 200ºC (3 min) and 200-270ºC (8ºC/min). Injector and detector temperatures were maintained at 220ºC and 290ºC, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of n-pentane, v/v) in the splitless mode. Helium was used as carrier gas with a flow rate of 1 mL/min.

Gas Chromatography-Mass Spectrometry (GC/MS):

GC-MS analyses of two oils were performed on a Agilent HP-5973 mass selective detector coupled with a Agilent HP-6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m × 0.25 mm i.d, film thickness, 250 nm) and operating under the same conditions as above was described .The flow rate of helium as carrier gas was 1 mL/min. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000.

Identification of bioactive and fragrant components:

Essential oils were analyzed by GC and GC/MS systems using a non-polar column and identification of components in these oils were based on retention indices (RI) relative to *n*-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature [33, 34]. The percentage composition of two samples was computed from the GC-FID peak areas without the use of correction factors.

3. Results and discussion

Leaves and fruits of *Ferula assa-foetida* yielded 0.043% and 0.49% (w/w) of dark yellow oils, respectively, which were determined by the gravimetric method and calculated as percentages with respect to the mass of starting dry plant materials. In these oils 23 and 25 components, which represented about 86.85% and 91.13% of the total composition, were identified for leaves and fruits oil, respectively, and listed in Table 1 with their percentage composition. The constituents are listed in order of their elution from HP-5MS column. trans-2-undecen-1-ol (17.26%), thymol (10.89%), dodecanal (9.70%), spathulenol (8.54%) and β-eudesmol (6.82%) were the major components in leaves oil and *γ*-elemene (32.21%), α-pinene (12.81%) and β-pinene (6.22%) were the major components in fruits oil.

Table 1. Bioactive and fragrance components of F. assa-foetida Leaves (A) and Fruits (B) from Iran

Compound[a]	**A, %**	**B, %**	**RI**[b]	**Compound**[a]	**A, %**	**B, %**	**RI**[b]
α-Pinene [M]	-	12.81	931	Dodecanal [M]	9.70	1.27	1407
β-Pinene [M]	-	6.22	972	*Trans*-Caryophyllene [S]	0.95	1.95	1413
Myrcene [M]	-	1.24	990	*γ*-Elemene [S]	-	32.21	1429
P-Cymene [M]	3.08	-	1022	*α*-Humulene [S]	-	0.95	1445
Limonene [M]	-	2.05	1025	*Trans*, 2-Dodecene-1- ol [M]	0.55	-	1468
Terpinolene [M]	-	2.85	1086	*Allo*-Aromadenderene [S]	-	0.89	1470
Allo-Ocimene [M]	-	0.98	1128	Germacrene D [S]	-	1.52	1473
Decanal [M]	2.76	-	1205	Dodecanol [M]	1.96	-	1476
Cis-Piperitone epoxide [M]	-	3.55	1237	*β*- Selinene [S]	-	1.07	1478
Carvacrol Methyl Ether [M]	0.96	-	1242	Bicyclogermacrene [S]	-	3.33	1490
Trans, 2-Decene-1- ol [M]	2.52	-	1270	*E,E*- Alpha-Farnesene [S]	-	1.29	1500
1-Decanol [M]	1.65	-	1276	*γ* – Cadinene [S]	-	0.87	1508
Thymol [M]	10.89	4.45	1293	*δ*- Cadinene [S]	-	1.14	1518
Carvacrol [M]	0.87	-	1302	Germacrene-B [S]	-	0.65	1550
Undecanal [M]	1.79	-	1306	Spathulenol [S]	8.54	-	1572
Bicycloelemene [S]	-	3.96	1334	Caryophyllene oxide [S]	4.38	-	1576
Undec-9z-en-1-al [M]	3.27	-	1338	Humulene,1,2-epoxide [S]	0.72	-	1602
α-Cubebene [S]	-	0.83	1347	Tetradecanal [S]	2.19	-	1611
α- Copaene [S]	-	3.77	1370	*β*- Eudesmol [S]	6.82	-	1643
Trans, 2-Undecene-1- ol [M]	17.26	-	1372	*α*- Eudesmol [S]	1.39	-	1646
Tetradecene <1-> [S]	-	0.81	1375	*α*- Muurolol [S]	1.94	-	1649
β- Bourbonene [S]	2.32	0.85	1381	Hexahydrofarnesyl acetate [S]	1.17	-	1843
β- Elemene [S]	-	3.16	1389	Phytol [D]	2.42	-	1922
				Total	90.12	94.68	

[a]Compounds listed in order of their RI.
[b]RI (retention index) measured relative to n-alkanes (c_8-c_{32}) on the non-polar HP-5MS column.
%, Relative percentage obtained from peak area.
[M] Monoterpene, [S] Sesquiterpene, [D] Diterpene.

In this part, we present biological properties and the application of some components from *Ferula assa-foetida* essential oils. These are as below:

***α*-Pinene:** is used as a fragrance substance to improve the odor of industrial products such as insecticides, antiseptics.

***β*-Pinene:** is used as a fragrance material in household perfumery and as antifungual, insecticides and polymer industries.

Myrcene: is used in the production of terpene polymers, terpene- phenol resins and terpenemaleate resins. Used as a solvent or diluting agent for dyes and varnishes.

***P*-Cymene:** is used to improve the odor of soaps and as a masking odor for industrial products, as an intermediate for musk perfumes, its addition to antiseptic preparations, as solvent for dyes and varnishes.

Limonene: is used as fragrance material for perfuming household products and as component of artificial essential oils.

***Allo*-Ocimene:** is used to a small extent in the perfume industry, used as a diluting agent for varnishes and dyes and as a component for terpene polymers.

Decanal: is used in fragrances and flavoring.

1-Decanol: is used in the manufacture of plasticizers, lubricants, surfactants and solvents.

Thymol: is used as disinfectant in oral hygiene products, starting material for the production of racemic menthol. Meanwhile it has antibacterial, antifungal and antiseptic activities.

Carvacrol: is used as disinfectant in organic synthesis, anti-infective, anthelmintic.

***α*- Copaene:** is used as a strongly attracting to an agricultural pest.

Dodecanal: is used in perfumery, added to aroma compositions to obtain citrus notes.

Germacrene D, B: it has antimicrobial and insecticidal properties.

Dodecanol: is used to make surfactants, lubricating oils, and pharmaceuticals. In cosmetics, and emollient.

Table 2. Bioactive And Fragrance Components Percentage Of Some Ferula Species Essential Oils

Plant Name	Major Components, %
F. elaeochytris fruit [18]	Nonane (27.1), *α*-Pinene (12.7), Germacrene B (10.3)
F. szowitsiana leaf [19]	*β*-Eudesmol (32.0), *α*-Eudesmol (18.2), *α*-Pinene (8.6)
F. szowitsiana aerial parts [20]	*α*-Pinene (12.6), Germacrene D (12.5), *β*-Pinene (10.1)
F. microcolea aerial parts [21]	*β*-Pinene (15.9), *α*-Pinene (10.4), *β*-Caryophyllene (8.6%)
F. hirtella aerial parts [22]	*α*-Pinene (15.4), Thymol (14.9)
F. stenocarpa aerial parts [23]	*α*-Pinene (48.8), *β*-Pinene (30.1)
F. galbaniflua stem and root [24]	*β*-Pinene (46.4 and 58.8, resp.)
F. flabelliloba aerial parts [25]	*δ*-Cadinene (13.2), *α*-Cadinol (12), *α*-Pinene (10)
F. ovina aerial parts [26]	*α*-Pinene (50.0), Limonene (11.5)
F. gummosa fruit [27]	*β*-Pinene (43.78), *α*-Pinene (27.27), Myrcene (3.37)
F. arrijonii leaf [28]	*β*-Phellandrene (16.6), Germacra-1(10),5-dien,11-ol- (15.4)
F. communis leaf [29]	Myrcene (53.5), Aristolene (8.5)
F. flabelliloba fruit [30]	10-epi-*γ*- Eudesmol (14.1), *β*-Dihydroagrofuran (13.3)
F. latisecta aerial parts [31]	(*Z*)-Ocimenone (32.4), (*E*)-Ocimenone (20.3)

According to a similar experiment on the essential oil from aerial parts of *F. assa-foetida* in the province of Kerman, south of Iran, in August 2005 [17], the major components were reported *E*-1-Propenyl sec-butyl disulfide (40.0%) and Germacrene B (7.8%), since, in our work, trans-2-undecen-1-ol (17.26%) and *γ*-elemene (32.21%) are presented as major components in oils from leaves and fruits, respectively, in Kashan species. As can be seen, the chemical composition of *F. assa-foetida* in our results is different from the other one. *e.g* Kashan species was no *E*-1-Propenyl sec-butyl disulfide, since, eight components: α-Pinene, β-Pinene, Myrcene, para-cymene, limonene, humulene, Gmma- cadinene, and Germacrene B, exists in both species above mentioned oils.

These differences between the oil compositions of one species of *Ferula* from two different areas can be related to climatological factors (from south to central Iran) and is discussed in phytological studies.

Previous studies on essential oils of members of *Ferula* genus showed various compositions. Table 2 shows the diversity of major components among oils of some *Ferula* species which have been analyzed by some investigators [18-31]. α and β-Pinene in *F. elaeochytris* [18], *F. szowitsiana* [19, 20], *F. microcolea* [21], *F. hirtella* [22], *F. stenocarpa* [23], *F. galbaniflua* [24], *F. flabelliloba* [25], *F. ovina* [26] and *F. gummosa* [27] oils are the major components. In the other hand, β-phellanderene in *F. arrijonii* [28], myrcene in *F. communis* [29], 10-epi-γ- eudesmol in *F. flabelliloba* [30] and (Z),(E)- ocimenone in *F. latisecta* [31] oils are major components. Thereby, in survey of these reports, it can be show significant differences for the oils, which can be attributed to either climatological factors or genetic differences of the plants and experimental conditions.

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