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Original article

Chemical Composition and Biological Activity of *Ferula aucheri* Essential Oil

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Abstract

Background and objectives: Antibiotics resistance and unpleasant side effects of AChE inhibitors have led to an increased interest in herbs as potential sources. *Ferula aucheri* (Syn: *Dorema aucheri*) an indigenous species of *Ferula* (Apiaceae) grows in Iran and is used as food and medicinal plant. The present study was aimed to identify the oil composition and evaluate antimicrobial and AChE inhibitory activity of flowering tops, fruits and roots. **Methods:** The chemical composition of the oils was recognized by GC and GC-MS. The antimicrobial effects were assessed on 12 microorganisms by disc diffusion and micro-well dilution methods and AChE inhibitory potential by a modified version of Ellman's method. **Results:** Sixty five compounds were identified from different organs and the notable characteristics have been high amounts of sesquiterpenes. Germacrene B (14.96%) and β-caryophyllene (12.87%) were distinguished as major components of flowering tops. Cis-dihydroagarofuran (9.02%) and δ-cadinene (8.28%) were identified as the remarkable constituents of fruit. δ-cadinene (18.25%) and gurjunene (12.62%) were detected from the roots by high content. All volatile oils exhibited lower MICs on *Bacillus subtilis*, *Klebsiella pneumonia*, *Shigella dysenteriae*, and *Salmonella paratyphi*-A serotype compared with gentamicin. Root and fruit oils were more effective than gentamicin against *Escherichia coli* and flowering tops oils proved lower MICs versus *Staphylococcus aureus*. Fruits and root oils showed weak potency for inhibiting AChE with IC_{50} values 554.05±4.65 and 239.69±3.5 µg/mL, respectively and flowering tops exhibited moderate activity (179.06±4.3 µg/mL). **Conclusion:** The findings demonstrated that *F. aucheri* essential oils possessed antimicrobial activities with inhibition properties toward AchE.

Keywords: AChE inhibitory activity; antimicrobial activity; *Dorema aucheri*; essential oil; *Ferula aucheri*

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Introduction

For centuries, essential oils have been widely employed as bactericide, virucide, fungicide, parasiticide, insecticide, and for other medicinal uses such as sedative, analgesic, spasmolytic, anti-inflammatory, and topical anesthetic properties. These applications have not changed much over time except that more information has been obtained about their mechanisms of action, especially at the antimicrobial level. Essential oils are complex mixtures of volatile molecules, including terpenoids, phenol-aromatic compounds, and aliphatic components [1].

Dorema, *Ferula* and *Leutea* are three genera of Apiaceae family from the subtribe of Ferulinae [2]. Since ancient times, several species of these genera have been the origins of oleo gum resins that were used in traditional medicine including asafoetida, sagapenum, galbanum and ammoniacum [3]. In recent years, morphological similarities, as well as presence of chemical

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constituents such as volatile aromatic acids and sesquiterpene lactones in these genera, have led to a closer examination of the phylogenetic relationships between them [2]. Following the clear correlation among *Dorema* and *Ferula* according to fruit anatomical, morphological and immunological data, as well as the molecular phylogeny findings, *Dormera* was transferred into *Ferula* genus [4,5].

The genus *Ferula* encompasses 180-185 species, the third largest genus of this family, and is mainly widespread in the Mediterranean and Central Asia [6]. Thirty species of *Ferula* genus are introduced in the Iranian flora of which some are endemic [7]. Species of *Ferula*, in Iranian traditional medicine, have been used for treating neurological disorders, inflammations, digestive disorders, malaria, and microbial diseases. Particularly, *Ferula* species have been recognized as a considerable source of antimicrobial compounds while some of them have not been reported from other plants [8,9].

Ferula aucheri (Boiss.) Piwczynski, Spalik, M.Panahi & Puchalka (Syn: *Dorema aucheri*) is one of the endemic species which grows in the beginning of spring in western mountains and southern provinces of Iran. The plant, locally known by the names "Bilhar" and "Kandal-e-koohi", is used by the local population for preparing native foods and also for its medicinal properties [10,11]. Traditionally, Oleo gum-resin obtained from the herb has been employed as an emmenagogue, expectorant, and tonic. The root of *F. aucheri* has been used as a burn healer [12]. Its leaves are consumed regularly in a great amount as vegetables and medicinally applied as an antiparasitic agent for the digestive system [13]. Most of the previous studies have been focused in the medical field including its toxicity and carcinogenicity, protective effects on the liver, stimulant, expectorant, antispasmodic, neuroprotective, anti-hypertensive, antidiabetic, hypolipidemic and antihypercholesterolaemic properties [12,14]. Limited investigation on essential oils of aerial parts and leaves of the plant demonstrated presence of a large group of sesquiterpene derivatives with potent antioxidant activities [12,15]. Former reports have indicated the antibacterial activity of the root and aerial parts extracts [10,16].

To the best of our knowledge, there is no investigation for assessing the content of essential oils of the root, fruits and flowering tops of *F. aucheri*. The present study was aimed to identify the essential oil composition, and evaluate some biological activity including antimicrobial and AChE inhibitory of the flowering tops, fruits and roots volatile oils of the plants.

Materials and Methods

Ethical considerations

Ethical approval for this study was granted by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran (ethics committee reference number: IR.TUMS.PSRC.REC.1396.2683).

Chemicals

The chemicals acetylthiocholine iodide (ATCI), acetylcholine esterase enzyme (AChE) from bovine erythrocytes, donepezil hydrochloride and sodium chloride were purchased from Sigma-Aldrich Chemical Company (Germany). All microbial culture media, anhydrous sodium sulfate, 5,5′ -Dithiobis-2-nitrobenzoic acid (DTNB) and other solvents were provided from Merck Chemical company (Germany).

Plant material

The flowering tops of *F. aucheri* were collected during the flowering stage in June 2017 and the fruits and roots were gathered in August and September of 2017, respectively from Hezar Mountain, Kerman, Iran. The identification was performed by a botanist, Dr. Yousef Ajani. The voucher specimen (7061-TEH) has been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Essential oil extraction

The shade-dried flowering tops, fruits, and roots of *F. aucheri* were ground and each powder (200 g) was subjected to hydrodistillation for 4 h at normal pressure using a Clevenger- type apparatus. The essential oils were collected, dehydrated over anhydrous sodium sulfate and finally stored in a sealed vial at 4 °C until GC/MS analysis and biological test as described subsequently.

GC and GC/MS analysis

Gas chromatography analysis of volatile components was performed on HP 7890 Network GC System (Agilent Technology), equipped with HP-5MS (DB-5) a fused silica column (30 m × 0.25 mm i.d., film thickness, 0.25 µm). The oven

temperature was programmed from 50 to 280 at a rate of 5 °C/min. The temperatures of 250 and 280 °C were used for the injector and the detector, respectively. Helium (99.999 %) was served as the carrier gas at a flow rate of 1 mL/min with a split ratio equal to 1/35. The oils were analyzed by GC/MS using a Hewlett Packard 5975 mass selective detector connected to an HP 7890 gas chromatograph at the above conditions. Mass spectra were taken at 70 eV ionization energy.

Identification of the components of the oils

Retention indices (RI) for all the components of *F. aucheri* were determined by co-injection of a homologous series of n-alkanes (C_8-C_{24}) and calculated according to the Van Den Dool and Kratz method [17]. The compounds were identified by direct comparison of retention indices with published data in the literature together with a comparison of their mass fragmentation patterns with MS library (Wiley7n.l) [18], Adams and NIST database [19].

Antibacterial activity determination

Bacterial strains

To assess the antibacterial properties of the test samples against a panel of pathogenic microorganisms, the following microbial strains were supplied by the Iranian Research Organization for Science and Technology (IROST). Nine bacterial strains including *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 29737), *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Proteus vulgaris* (PTCC 1182), *Salmonella paratyphi* A-serotype (ATCC 5702), as well as two fungus including *Aspergillus brasiliensis* (ATCC 5011) and *Aspergillus niger* (ATCC 16404), and a yeast: *Candida albicans* (ATCC 10231) were also employed in the present research.

Disc diffusion method

As a preliminary step, the disc diffusion assay was accomplished for the determination of antimicrobial activity of essential oils [20]. For sterilization, essential oils of *F. aucheri* were filtered through 0.45 µm millipore filters. Respectively, 100 µL suspension of each tested microorganism including 10^8 CFU/mL of bacteria, 10^4 spore/mL of molds and 10^6 CFU/mL of yeast were overspread using a sterile spreader on the nutrient agar (NA), potato dextrose (PD) agar and sabouraud dextrose (SD) agar mediums. The impregnated discs (6 mm in diameter) with 10 µL of the essential oils were placed on the inoculated agar medium. 5% DMSO was used as the negative control. All plates were incubated for 24 h at 37 °C for bacterial strains and at 30 °C for yeast and mold. Two reference antibiotics Gentamicin (10 µg/disc) and rifampin (5 µg/disc) were used as the positive controls for tested bacteria and nystatin (100 I.U./disc) for fungi. The antibacterial activity was evaluated by measuring the diameter of inhibition zones (IZ) in millimeters and each assay was performed in triplicate. Diameters of inhibition zones were declared as Mean ± standared deviation (SD).

Micro-well dilution assay

Ferula aucheri essential oils were employed to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for microbial strains using micro-well dilution assay [21]. In this manner, the 96-well plates were prepared by dispensing 95 µL of the nutrient broth and 5 µL of the inoculum into each well. The suspensions of microorganism strains were made from 12 h broth cultures and the turbidity was adjusted to 0.5 McFarland scale. The procedure involved preparing two-fold dilutions of oil samples (7.8-1000 µg/mL) in sterile test tubes including brain heart infusion (BHI) broth for bacteria and sabouraud dextrose (SD) broth for yeast. The well containing 195 µL of the nutrient broth without the test materials and 5 µL of the inoculum on each strain was used as the negative control. Three antibiotics were employed in the same conditions as described to test samples as positive controls including gentamicin and rifampin for bacteria and nystatin for fungi. The plate was covered with a sterile plate sealer. The content of wells were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. At the end of the incubation period, the plates were evaluated for microbial growth by the presence of a white pellet at bottom of the well and by plating 5 µL samples from clear wells on nutrient agar medium. MIC values were considered as the lowest concentration of the sample that prevented the visible growth of microorganisms as compared to the control well (grown without

essential oil). MBC (minimum bactericidal concentration) is usually an extension from the MIC, whereas the value was determined by a subculture of 10 µL from the wells medium with no visible growth and turbidity onto agar plates. After incubation, when no viable organism appeared in the culture, the sample denoted a bactericidal action [22]. The experiments were repeated at least three times for each microorganism.

Acetylcholinesterase inhibitory activity

AChE inhibitory activity of the essential oils was measured in a 96-well microplate based on Ellman's method with some modifications [23]. The basis of this method is to determine the rate of thiocholine production as an enzyme-catalyzed product on acetylthiocholine as a substrate. Afterwards, thiocholine reacts with Ellman's reagent (DTNB) to give 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate. The color intensity can be detected by an ELISA reader. In short, 25 µL of ATCI (15 mM), 125 µL of DTNB (3 mM), 25 µL of ATCI (15 mM), 50 µL of phosphate buffer (pH 7.9) and 25 µL of the essential oil solution at various concentrations (37.5-600 µg/mL, in methanol) were added in each well of separate 96-well microplates. The absorbance was read at 405 nm every 13 s for 65 s. Then, 25 µL of AChE enzyme in phosphate buffer (0.22 U/mL) was added to the wells and absorbance was again recorded in 13 s intervals for 104 s. Finally, the absorption curve was plotted against time and the slope of the line was calculated that indicated the amount of the enzyme activity. The percentage of enzyme inhibition was evaluated by comparing rates of reaction of samples relative to blank (methanol without test sample). The experiments were done in triplicate and the results were reported as Mean±SD. It should be noted that in this test, donepezil, a medicine with potent AChE inhibitory activity, was utilized as the positive control.

Result and Discussion

The qualitative and quantitative essential oil compositions considering their elution order on the HP-5MS column have been listed in table 1. In total, sixty-five compounds were identified as the result of GC and GC/MS analysis of the essential oils of flowering tops, fruits and roots of *F. aucheri*. The classification of the recognized compounds, based on the main groups has been summarized at the end of table 1.

The yields of essential oils were 0.27, 0.18 and 0.18 % (w/w), respectively, for the flowering tops, fruits and roots of *F. aucheri*, calculated on a dry weight basis. The samples of oils obtained from hydrodistillation were pale yellow with a distinct sharp odor. Fourty-seven compounds were identified from the essential oil of flowering tops, which accounted for 97.53% of the total essential oil. The classification of the known compounds demonstrated that the essential oil predominantly involved sesquiterpene hydrocarbons (61.9%). The flowering tops oil was characterized by the absence of nonterpene hydrocarbons and appearance of a diterpene compound namely neophytadiene (0.18%). Germacrene B (14.96%) and β-caryophyllene (12.87%) were distinguished as the major components of the flowering tops oil. Thirty-six compounds were recognized from the essential oil of fruit that included 91.39% of total essential oil. The results showed that sesquiterpene hydrocarbons (56.17%) were the main constituent group while non-terpenes (0.76%) and oxygenated monoterpenes (0.91%) covered only a small percentage of the oil content. Cis-dihydroagarofuran (9.02%), 2-pentadecanone (8.98%), δ-cadinene (8.28%) and γ-cadinene (8.17%) were determined as the remarkable constituents of the fruit oil. Thirty-nine compounds representing 91.87% of the oil contents were identified in the root. The essential oil from the root was described by a significant amount of sesquiterpene hydrocarbons (69.09%) and a scant value of non-terpene hydrocarbons (0.19%). The oil was clarified by a high content of δ-cadinene (18.27%), α-gurjunene (12.63%), and α-selinene (9.22%).

The analysis of current research indicated the presence of a large group of sesquiterpene derivatives which was in agreement with the outcomes obtained from prior studies. Nineteen compounds were observed in all oil samples. δ-Cadinene, α-gurjunene, and α-selinene were recognized as chief components.

Table 1. Chemical compositions of the flowering tops, fruits and roots essential oils of *Ferula aucheri*

No.	Compounds	Calc. RI	Rep. RI	RT	Flowering tops (%)	Fruits (%)	Roots (%)
1	α-Pinene	930	932	8.63	3.54	4.51	0.24
2	Camphene	944	946	9.16	0.47	0.41	0.12
3	p-Methylethylbenzene	952	956	9.67	-	0.12	-
4	β-Pinene	973	974	10.18	0.51	-	-
5	Sulcatone	977	981	10.65	0.13	0.22	0.10
6	2-Pentylfuran	983	984	10.72	-	-	0.46
7	β-Myrcene	989	988	10.75	1.02	-	-
8	Mesitylene	993	994	10.79	-	0.64	-
9	δ-2-Carene	1000	1001	11.03	-	-	0.23
10	α-Phellandrene	1003	1002	11.17	0.17	-	0.33
11	α-Terpinene	1016	1014	11.60	0.17	-	0.16
12	o-Cymene	1020	1022	11.89	0.54	0.41	0.23
13	Limonene	1023	1024	12.03	2.84	1.05	-
14	β-Phellandrene	1025	1025	12.04	-	-	1.72
15	cis-β-Ocimene	1031	1032	12.40	0.46	0.19	-
16	2-Heptyl acetate	1037	1038	12.61	-	-	0.63
17	trans-β-Ocimene	1046	1044	12.76	3.50	-	-
18	γ-Terpinene	1053	1054	13.08	0.86	-	0.17
19	α-Terpinolene	1085	1086	14.07	1.65	0.20	-
20	p-menth-2-en-1-ol	1134	1136	15.17	-	-	0.23
21	Thymol methyl ether	1236	1232	18.63	0.13	0.26	0.97
22	l-Bornyl acetate	1282	1284	20.14	0.29	0.65	2.58
23	Thymol	1288	1289	20.32	-	-	0.19
24	α-Cubebene	1344	1345	21.85	-	-	0.14
25	α-Copaene	1376	1374	22.57	1.57	3.10	5.24
26	β-Elemene	1387	1389	22.99	0.20	-	-
27	Jasmone	1394	1392	23.18	0.26	-	-
28	α-Gurjunene	1408	1409	23.46	2.33	6.82	12.63
29	β-Funebrene	1411	1413	23.58	-	0.32	-
30	β-Caryophyllene	1419	1417	23.72	12.87	3.78	-
31	β-Gurjunene	1429	1431	23.94	-	-	0.45
32	γ-Elemene	1432	1434	24.06	4.81	-	-
33	α-Guaiene	1437	1437	24.12	-	1.60	5.99
34	Aromadendrene	1442	1439	24.23	1.67	2.34	4.49
35	α-Humulene	1453	1452	24.59	1.46	0.41	-
36	α-Amorphene	1480	1483	25.13	1.28	2.05	0.83
37	Germacrene D	1484	1484	25.28	0.44	-	-
38	β-Selinene	1489	1489	25.43	2.67	7.80	5.76
39	2-Tridecanone	1492	1495	25.57	0.87	-	-
40	α-Selinene	1496	1498	25.64	3.65	5.68	9.22
41	α-Muurolene	1501	1500	25.75	1.26	1.87	2.19
42	β-Bisabolene	1506	1505	25.90	1.01	0.86	1.03
43	γ-Cadinene	1512	1513	26.11	4.70	8.17	-
44	cis-Dihydroagarofuran	1518	1519	26.24	4.64	9.02	-
45	δ-Cadinene	1523	1522	26.32	4.70	8.28	18.27
46	trans-Cadina-1,4-diene	1532	1533	26.53	0.53	-	1.60
47	α-Cadinene	1536	1537	26.64	0.66	1.10	0.94
48	α-Calacorene	1543	1544	26.76	-	0.94	0.31
49	Selina-3,7(11)-diene	1547	1545	26.77	1.13	-	-
50	Elemicin	1553	1555	27.04	-	0.58	-
51	Germacrene B	1557	1559	27.13	14.96	1.05	-
52	trans-Nerolidol	1564	1561	27.21	-	2.08	1.24
53	Viridiflorol	1591	1592	27.78	1.64	-	-
54	Rosifoliol	1604	1600	28.07	3.98	0.58	4.70
55	γ-Eudesmol	1633	1630	28.87	0.73	1.99	1.10
56	α-Cadinol	1654	1652	29.38	2.78	2.78	0.40
57	E-9-Tetradecenol	1666	1667	29.92	-	-	0.61
58	2-Pentadecanone	1698	1697	30.27	2.63	8.98	5.85
59	Farnesol	1711	1712	30.76	0.42	0.55	-
60	(2Z,6E)-Farnesal	1724	1722	30.86	0.26	-	-
61	Neophytadiene	1801	1800	32.68	0.18	-	-
62	E-11-Tetradecenyl acetate	1814	1810	32.88	-	-	0.19
63	1-Nonadecene	1891	1892	34.96	-	-	0.19

Table 1. Continued

No.	Compounds	Calc. RI	Rep. RI	RT	Flowering tops (%)	Fruits (%)	Roots (%)
64	Hexadecanoic acid	1958	1959	35.64	0.85	-	0.14
65	Ethyl palmitate	1973	1975	36.24	0.11	-	-
	Monoterpene hydrocarbons				15.73	6.77	3.20
	Oxygenated monoterpenes				0.68	0.91	3.97
	Sesquiterpene hydrocarbons				61.9	56.17	69.09
	Oxygenated sesquiterpenes				14.45	17	7.44
	Diterpene hydrocarbons				0.18	0.00	0.00
	Non-terpene hydrocarbons				0.00	0.76	0.19
	Oxygenated non-terpenes				4.59	9.78	7.98
	Non-oxygenated constituents				77.81	63.70	72.48
	Oxygenated constituents				19.72	27.69	19.39
	Total identified constituents				97.53	91.39	91.87

Cal. RI: Calculated retention indices; Rep. RI: Reported retention indices

Table 2. Comparing essential oil constituents of *Ferula aucheri* with some other Iranian *Ferula* species

Plant name	Part	Main compounds (%)	References
Ferula aucheri	FT[d]	Germacrene B (14.96), β-Caryophyllene (12.87)	[Present work]
D[a]. ammoniacum	FL[e]	δ-Cadinene (11.58), α-Himachalene (7.71), α-Pinene (6.37)	[25]
F. cupularis	FL	Limonene (25.04), d-2-Carene (15.81), Sabinene (7.96)	[26]
F. aucheri	FR[f]	cis-Dihydroagarofuran (9.02), δ-Cadinene (8.28), γ-Cadinene (8.17)	[Present work]
D. ammoniacum	FR	(Z)-Ocimenone (22.3), (E)-Ocimenone (18.1)	[27]
F. gummosa	FR	β-Pinene (43.8), α-Pinene (27.3)	[28]
F. badrakema	FR	β-Pinene (45.8), α-Pinene (10.9)	[7]
F. aucheri	R[g]	δ-Cadinene (18.27), α-Gurjunene (12.63), α-Selinene (9.22)	[Present work]
D. ammoniacum	R	3-n-Butyl phthalide (62.49), Benzyl butanoate (6.57), Liguloxide(5.15)	[25]
D. ammoniacum	R	β-Bisabolene (15.1), Hexadecanal (13.2), (E)-Nerolidol (11.3)	[29]
D[b]. glabrum	R	Myristicin (14.1), Elemicin (11.7)	[30]
D. glabrum	R	δ-Cadinene (12.8), β-Bisabolene (7.5)	[31]
F. persica	R	Dimethyl trisulfide (18.2) ,Myristicin (8.9), Dimethyl tetrasulfide (7.6)	[24]
F. gummosa	R	β-Pinene (58.8)	[24]
F. haussknechtii	R	α-Pinene (16.9), Isoverbanol (15.19), Camphene (19.05)	[24]
F. alliacea	R	10-epi-g-Eudesmol (22.3), Valerianol (12.5), Hinesol (8.3)	[24]
D[c]. aucheri	AE[h]	α-Eudesmol (31.2), δ-Cadinene (10.9), β-Caryophyllene (4.9)	[17]
D. ammoniacum	AE	β-Himachalene (9.3), β-Chamigrene (8.7)	[29]
D. glabrum	AE	Elemicin (38.6), Myristicin (14.3)	[32]
F. persica	AE	Dillapiole (57.3), Elemicin (5.6), Limonene (4.4)	[24]
F. haussknechtii	AE	Camphene (19.87),α-Pinene(15.12), Isoverbanol (15.01)	[24]
D. aucheri	LV[i]	β-Caryophyllene (7.17-35.73), Thymol (23.45-29.64)	[13]
D. aucheri	LV	Curzerene (18.7), Spathulenil (6.68), Gemberen (6.66)	[33]
D. aucheri	LV	α-Eudesmol (39.2), δ-Cadinene (12.9)	[34]
F. cupularis	LV	β-Pinene (13.87), β-Ocimene (9.05)	[26]

a, b, c: The previous name of these plants have been used as mentioned in the related articles and now they have been transferred to the *Ferula* genus, d: flowering tops, e: flowers, f: fruits, g: roots, h: aerial parts, i: leaves

Although a qualitative difference among the oils from the flowering aerial part, fruit, and root was obvious, the flowering top and fruit oils exhibited more similarity in their chemical profile. Twenty-eight components were similar in both flowering tops and fruit oils.According to the result of previous research, among 35 constituents from air-dried aerial parts, representing 89.2% of the total detected components, α-eudesmol (31.20%) and δ-cadinene (10.90%) were the major compounds and in the next rating 2-pentadecanone (5.90%) and β-caryophyllene (4.90%) assigned [15]. Also, the results of another earlier study on leaves essential oil, just before the flowering stage revealed the following compounds as the main constituents of the oil including β-caryophyllene, thymol, β-gurjunene, carvacrol, and cuparene [12].

In table 2, the essential oils compounds of *F. aucheri* have been compared with some other plants from the *Ferula* genus. According to the literature, α-pinene and β-pinene are mainly observed in the essential oils of *Ferula* and used as a chemotypic marker for this genus [24]. In the present work, the above-mentioned compounds have also been recognized, although it should be noted that they were not major constituents. Previous studies reported that geographic factors such as climate, soil condition, and altitude can significantly influence essential oil yield and chemical profile of medicinal plants. Stresses induced by microorganism and insects,

harvesting time, drying and storage quality, besides essential oil extraction method could be regarded as effective factors on remarked differences [21].

In developing countries, the main cause of morbidity and mortality is associated with pathogenic microorganisms. Nowadays, antibiotic resistance is known as one of the greatest threats to global health. To overcome this problem, researchers have paid special attention for discovering antimicrobial agents, particularly from natural sources. Until now, there are more than 1340 plants with determined antimicrobial activities, and over 30,000 antimicrobial components have been isolated from plants. The antimicrobial impact of essential oils and its various components extracted from medicinal plants has been well documented [9].

The results of the antibacterial activity evaluation of the essential oils have been manifested in table 3. As observed in the table, the essential oils of *F. aucheri* in disc diffusion assay were inactive against all microorganisms and only exhibited a weak (<10 mm) to moderate activity (10-15 mm) for three Gram-positive bacteria including *B. subtilis*, *S. epidermidis* and *S. aureus*. Among the essential oils, the oils obtained from root exhibited lower MICs against bacterial strains such as *B. subtilis*, *E. coli*, *K. pneumonia*, *S. dysenteriae* and *S. paratyphi*-A compared with gentamicin and were more effective than rifampin on *E. coli*, *S. aureus* and *S. paratyphi*-A. Antibacterial activity of the fruits volatile oils versus *K. pneumonia*, *S. paratyphi-A*, *B. subtilis*, *S. dysenteriae* and *E. coli* were greater than positive control gentamicin. In comparison with rifampin, fruits oils were more effective on *S. paratyphi*-A, *K. pneumonia* and *S. dysenteriae*. The essential oils obtained from the flowering tops exhibited lower MICs against bacterial strains such as *S. paratyphi*-A, *K. pneumonia*, *B. subtilis*, *S. aureus* and *S. dysenteriae* compared with gentamicin. Antibacterial activity oils of the flowering tops were greater than rifampin on *S. paratyphi*-A, *K. pneumonia*, *S. aureus* and *S.* dysenteriae.

The lowest minimum inhibitory concentration of 31.25 μg/mL was observed for a Gram-positive strain *B. subtilis* with essential oil of root. The essential oils obtained from the fruit and flowering tops which were most similar in their chemical profile, particularly have showed more effect on two Gram-negative bacteria including *S. paratyphi-A* and *K. pneumonia*.

Former reports about the antibacterial activity of *F. aucheri* root and aerial part extracts have indicated that this plant as a potent antibacterial agent which inhibited the growth of a wide range of bacteria, remarkably against Gram-positive strains such as *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* better than Gram-negative strains [35,16]. *Shigella dysenteriae* a Gram-negative strain was found as the most sensitive microorganism against the essential oil of *D. ammoniacum* aerial parts with inhibition zones of 17 and MIC value and 250 μg/mL [29]. Also, the essential oil of *D. ammoniacum* fruits has been reported to possess significant antimicrobial activity against *B. subtilis*, *S. epidermidis* and *S. aureus* with inhibition zones of 23, 22, 17 mm and MIC values of 3.75, 3.75 and 7.5 mg/mL, respectively [27]. Results of antibacterial investigation on root and aerial parts of *F. haussknechtii essential* revealed that the essential oils were efficient on *Bacillus pumilus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (17-18 mm, 7.5 mg/mL) [24].

Ordinarily, Gram-negative bacteria are found to be more resistant to the effect of essential oils than Gram-positive bacteria. The reason for this resistance returns to their hydrophobic cell wall structure. This structure is mainly formed by lipopolysaccharides that prevent the penetration of hydrophobic constituents into the oil. However, as observed, essential oils of different parts of this herb showed growth inhibitory effects on both Gram-positive and Gram-negative bacteria.

Among the potential antibacterial medicines recognized, 75% were terpenes. Recently, it was announced that 67% of powerful constituents belong to monoterpenes and sesquiterpenes [36]. And also, several studies have revealed that aldehydes or phenolic compounds in essential oils showed high antibacterial activity followed by terpene alcohol [37].

According to these facts, essential oil of flowering tops due to a higher content of monoterpene compounds with proven antimicrobial effects such as α-pinene, β-pinene, limonene, and camphene, as well as the presence of a higher percentage of terpene alcohols and aldehydes, exhibited a greater inhibitory effect on bacterial growth.

Table 3. Antibacterial and antifungal activities of the flowering tops, fruits and roots of *Ferula aucheri* essential oils

Microorganism	Root			Fruit			Flowering tops			Rifampin[a]		Gentamicin[b]		Nystatin[c]	
	IZ[d]	MIC[e]	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	IZ	MIC	IZ	MIC
Staphylococcus aureus[p]	12	250	1000	9	250	>2000	-	125	2000	7	250	22	250	NA	NA
Bacillus subtilis[p]	-	31.25	1000	-	125	2000	9	125	2000	13	15.6	21	500	NA	NA
Staphylococcus epidermidis[p]	-	-	-	-	-	-	12	500	1000	11	500	21	500	NA	NA
Salmonella paratyphi-A[n]	-	250	1000	-	62.5	1000	-	62.5	1000	-	-	21	500	NA	NA
Escherichia coli[n]	-	125	2000	-	250	>2000	-	500	1000	10	250	21	500	NA	NA
Klebsiella pneumonia[n]	-	250	1000	-	62.5	2000	-	62.5	2000	40	250	35	500	NA	NA
Shigella dysenteriae[n]	-	250	1000	-	125	2000	-	125	500	8	250	18	500	NA	NA
Proteus vulgaris[n]	-	-	-	-	-	-	-	-	-	10	125	23	500	NA	NA
Pseudomonas aeruginosa[n]	-	-	-	-	-	-	-	-	-	-	-	8	500	NA	NA
Candida albicans	-	-	-	-	-	-	-	-	-	NA	NA	NA	NA	33	125
Aspergillus niger	-	-	-	-	-	-	-	-	-	NA	NA	NA	NA	27	31.2
Aspergillus brasiliensis	-	-	-	-	-	-	-	-	-	NA	NA	NA	NA	30	31.2

a: rifampin (5 µg/disc), b: gentamicin (10 µg/disc), c: nystatin (100 i.u./disc), d: inhibition zone in diameter (mm) around the impregnated discs including diameter of the disc (6 mm) [weak activity (<10 mm), moderate activity (10–15 mm), strong activity (15–20 mm), very strong activity (>20 mm)], e: minimal inhibition concentrations as µg/mL, NA: not applicable, p: Gram-positive strain, n: Gram-negative strain.

However, as the above-mentioned compounds were not the main component of the essential oils, the differences were not very considerable.Alzheimer's disease (AD) is the main reason for dementia accounting for more than 75% of cases. Presently, AChE inhibitors are the principal category of drugs for remedy. To date, numerous plant species that contain various categories of natural compounds including coumarins, alkaloids, terpenes, and polyphenols have been assessed for their AChE inhibitory activity and considered as potential candidates for innovative anti-AD drugs [38].

The results of AChE inhibitory activity which was carried out on essential oils from different organs of *F. aucheri* were compiled in table 4. Essential oils from the flowering tops exhibited moderate AChE inhibitory activity (IC_{50} = 179.06±0.03 µg/mL), fruits and roots oil showed weak potency for inhibiting AChE with the IC_{50} value of (554.05±0.06 and 239.69±0.08 µg/mL, respectively) in comparison with donepezil as an AChE inhibitor (IC_{50}= 0.026±0.002 µg/mL). Several investigations have demonstrated the inhibitory effects of monoterpenes on AChE because of their low molecular weight and lipophilic nature; thus, allowing them easily cross the blood-brain barrier such as α-pinene with notable inhibitory effects [39]. The results of the AChE inhibitory activity of sesquiterpenes indicated that if tested separately, show poor activity. A mixture of sesquiterpenes exhibited significant AChE inhibition activity, indicating that they act synergistically and enhance each other's effect [40]. As shown in table 1, the content of sesquiterpenes in root was higher than flowering tops and in flowering tops more than the fruit. Accordingly, from the two described aspects, the AChE inhibitory effects of flowering tops can be justified.

The weak activity of the essential oil of the fruit can be interpreted from the evidences in former reports that there could be antagonistic relationships between monoterpenoids and sesquiterpenes as well as monoterpenes and sesquiterpenes. In the fruit, the ratio of these compounds was close which may provide antagonistic interactions [39,42].

Table 4. AChE inhibitory properties of the flowering tops, fruits and roots of *Ferula aucheri* essential oil

Sample/Essential oil	AChE inhibitory activity
	IC_{50} (µg/mL)
Flowering tops	179.06±0.03
Roots	239.69±0.08
Fruits	554.05±0.06
Positive control (Donepezil)	0.026±0.004

To the best of our knowledge, the present research was the first report about the antimicrobial and AChE inhibitory properties of the essential oils obtained from different parts of *F. aucheri.* In conclusion, the results of this study suggest the essential oils of *F. aucheri* as

potential source of natural anticholinesterase and antibacterial agents. Potent antibacterial activity of *F. aucheri* essential oils, make them appropriate options to be used as natural flavors and preservatives in food and pharmaceutical industries; however, further toxicological studies are necessary to evaluate safety perspectives.

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Author contributions

Sheyda Ahmadi Koulaei performed plant preparation, extraction, and explanation of oil substances, biological tests and drafted the manuscript. Abbas Hadjiakhoondi and Zahra Tofighi advised the project. Mohammad Reza Delnavazi was the adviser for the project and managed the preparation of the essential oil. Yousef Ajani collected and identified the plant. Fatemeh Kiashi cooperated in the practical work. Narguess Yassa supervised the Ph.D. student and verified the accuracy of the data.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

GC/MS: gas chromatography/ mass spectrometry; AChE: acetylcholine esterase; SDS: sodium dodecyl sulfate; IC_{50}: the half maximal inhibitory concentration; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; RI: Retention indices; RT: Retention time; ATCI: acetylthiocholine iodide; DTNB: 5,5′ -Dithiobis-2-nitrobenzoic acid; I%: inhibition percentage; IROST: Iranian Research Organization for Science and Technology; NA: nutrient agar; PD: potato dextrose; SD: sabouraud dextrose; IZ: inhibition zones; BHI: brain heart infusion; AD: Alzheimer's disease