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Original article

Cytotoxic, Anti-Microbial and Anti-Alzheimer Potential of *Ferulago stellata* Boiss. Fruits and Its Chemical Composition

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Abstract

Background and objectives: The *Ferulago* species are perennial aromatic plants and have been used as digestive, flavoring, sedatives, and aphrodisiac agent. **Methods**: The volatile oil from *Ferulago stellata* fruits was attained through hydrodistillation methods using a Clevenger-type apparatus. Then, the chemical composition was assessed by gas chromatography mass spectrometry (GC-MS). The antioxidant activity of the volatile oil was evaluated by the DPPH method. The extracts were sequentially obtained by the Soxhlet apparatus with different solvents. To analyze the fatty acid profile, the non-polar extract (*n*-hexane) was converted to the corresponding methyl ester via saponification and esterification and assessed by GC. The antimicrobial activity of the volatile oil and extracts were evaluated against eight microorganisms and MIC and MBC were attained by the micro broth dilution method. The antiproliferative and acetylcholinesterase inhibitory activity of the extracts was evaluated as well. **Results:** Fourteen volatile constituents were identified of which the main constituent was 2,4,6-trimethyl benzaldehyde (56.05%). The essential oil indicated high antioxidant activity with IC_{50} value of 6.05 ± 0.77 µg/mL. The volatile oil showed powerful antimicrobial activity against all strains of the microorganisms with MIC 0.78 to 3.12 µg/mL and MBC between 1.56 and 6.25 µg/mL. The n-hexane extract showed higher cytotoxicity effect compared to other extracts. The result of the fatty acid profile demonstrated that the fruits were rich source of unsaturated fatty acids. **Conclusion:** *F. stellata* might be a potent source of antioxidant, antimicrobial, and healthy fatty acid which can be used in the pharmaceutical, cosmetic, and food industries.

Keywords: acetylcholinesterase; antioxidant; essential oil; fatty acid; *Ferulago stellata*

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Introduction

The genus *Ferulago* W. Koch. (called "Çakşır" or "Çağşır" in Turkey, and "Chavil" or "Chavir" in Persian) is a member of the Apiaceae family, including 49 species that are frequently growing in some parts of Asia, Africa, and Europe [1,2]. The genus *Ferulago* is distributed in Iran with

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eight species of which three are endemic. Thirty-four species exist in Turkey among which eighteen are endemic [3]. The *Ferulago* species are broadly employed in traditional medicine for their sedative, aphrodisiac, digestive and tonic properties and in cases of snake bites, intestinal worms, and headache [4]. It was used as a natural preservative to prolong the expiration date of meat, oil ghee, and dairy and gives them a pleasing taste. *Ferulago stellata* is a perennial shrub that grows in the west of Iran producing yellow flowers. It grows at the height of around 50–190 cm at an altitude of 1500–2150 m above the sea level as an endemic plant to Iran [5]. So far, the phytochemical properties of essential oils of several species including *F. sandrasica*, *F. pauciradiata*, *F. cassia*, *F. angulata*, *F. contracta*, *F. macrocarpa*, and *F. capillaris* have been investigated and these species possess potential activities such as antifungal [6], antibacterial [7,8], anticholinesterase [9,10] and antioxidant [7,10,11]. Phytochemical investigations have revealed that the main constituents of the essential oils of many *Ferulago* species contain α-pinene, 2,3,6-trimethyl benzaldehyde, p-Cymene, α-phellandrene, cis-chrysanthenyl acetate, terpinolene, sabinene, limonene, (Z)-β-ocimene, myrcene, δ-cadinene, and nonacosane [3]. To evaluate the effects of the *F. stellata* essential oil as a prospective antimicrobial and antioxidant agent in the pharmaceutical and food industries, the phytochemical property of the volatile oil has been investigated in the present study. Antimicrobial, anticholinesterase, and antiproliferative activity of the extracts were evaluated as well. The fruits contain lipid components that not only present flavor and aroma characteristics, but also produce nutritional value for the plant [12]. The fatty acid content of vegetable oil, which varies greatly based on the plant species, determines its suitability for a specific use such as medicinal, pharmaceutical, and industrial application [13]. In this work, we attempted to investigate the fatty acid composition of the fruits from *F. stellata* which has not been documented up to date. To the best of our knowledge, there is no report considering volatile constituents of the fruit, fatty acid profile, antimicrobial, and antioxidant effects of this plant; therefore, this study aimed to evaluate the chemical compositions, antioxidant, and antimicrobial activities of the volatile oil and antimicrobial, anticholinesterase and antiproliferative activity of the fruit extracts.

Materials and Methods

Ethical considerations

This study was approved by the Ethics Committee of Tabriz University of Medical sciences (IR.TBZMED.VCR.REC.1398.385).

Chemicals

MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], 2,2-diphenyl-1-picrylhydrazyl (DPPH powder), Mueller-Hinton agar, Mueller-Hinton broth, and Acetylcholinesterase (AChE) was purchased from Sigma (Germany). Galantamine hydrobromide, and Doxorubicin obtained from (Sigma-Aldrich (Germany), and Adriablastina (Italy) respectively. DTNB (5,5′-Dithiobis (2-nitrobenzoic acid) provided from Merck (Germany). All other chemicals and solvents were of analytical grade.

Plant material

Ferulago stellata Boiss. fruits were collected (about 1 kg) at the sides of the road around Saqez to Marivan, Iran (36° 09' 26.5" N, 46° 19' 17.1" E,1725 H) in June 2019. The plant was recognized by Dr. Ebrahimi, a botanist of the Faculty of Pharmacy, Tabriz University of Medical Science. A voucher specimen (TBZFPH 4057) was kept at the Tabriz University of Medical Science Herbarium. All solvents and reagents were of analytical grade.

Isolation of the essential oil

The crushed fresh fruits (100 g) were subjected to hydrodistillation with a Clevenger-type apparatus for 3 hours. The oil was stored at -18°C for further investigations.

Extraction

The crushed fruits (100 g) were sequentially extracted by Soxhlet apparatus with n-hexane, chloroform, ethyl acetate, and methanol at room temperature for 8 h. The extracts were filtered and dried with a rotary evaporator at 40 °C. The extracts were kept at -18 °C for further assessments.

Analysis of essential oil composition by GC/MS

The analysis of the volatile oil was carried out via a Shimadzu GC-MS-QP5050A, equipped with flame ionization detector (FID), and DB-1 capillary column (60 m × 0.25 mm id, 0.25 μm film thickness). Operating conditions were as

follows: carrier gas, helium with a flow rate of 1.3 mL/min; column temperature, 2 min in 50 °C, 50-275 °C at 3.0 °C/min and finally 3 min in 275 °C, injector temperature, 250 °C; detector temperature, 280 °C; volume injected, 1µL; split ratio 1:20. Mass spectra were taken at 70 eV over the mass range 30–600 amu, ion source temperature at 260 °C, and solvent delay 2.0 min. Identification of the oil constituents was based on the retention indices of n-alkanes (C8–C24) that were injected at the same condition to the GS-Mass and computer matching with NIST, the Wiley library, and compared to their mass spectra with those stated in the literature [14-23].

Preparation of FAME (fatty acid methyl ester)

The n-hexane extract was converted to the corresponding methyl ester via saponification and esterification. Ten mg oil was mixed with 0.5 ml n-hexane and 2 mLl of 2 M potassium hydroxide in methanol and vortexed for 2 min at room temperature. The sample was heated in a water bath at 60 ºC for 15 minutes and then it cooled with tap water. After that, 2 mL of NaCl (20% w/v), and 0.5 mL n-hexane were added to the tube and vortexed again, and centrifuged for 10 minutes. Finally, after 5 minutes, 1 µL of the upper layer of the tube was injected into the GC [24].

Analysis of fatty acid by GC

The chromatograph was equipped with a YL6100 GC (60 m × 0.25 mm, 0.2µm) capillary column (Teknokroma, Spain) and a flame ionization detector. The temperature program for the column started at 80 °C first, increased by 20 °C/min to 120 °C, and increased at 3 °C/min to 260 °C, and then hold at 260 °C for 10 min. The injector temperature was 260 ºC and the detector temperature was 280 ºC. The split ratio was 1:50 and the flow rate of the carrier gas (helium) was 2 mL/min.

Antioxidant activity

The free radical scavenging activity of the essential oil was investigated via DPPH (2,2-diphenyl-1- picrylhydrazyl) technique described by Heshmati Afshar et al. [25]. Fifty µL of different concentrations in methanol (1.25, 2.5, 5, 10. 20, and 100 mg/mL) were mixed with five mL of a 0.004% methanol solution of the DPPH. The absorbance of the sample was recorded at 517 nm after 30 minutes of dark incubation at room temperature. Quercetin and methanol were used as the reference standard and blank test, respectively. The percent of DPPH radical scavenging activity of essential oil was measured as follows:

$$I\,(\%) = \frac{\text{Abs blank - Abs sample}}{\text{Abs blank}} \times 100$$

Where Abs blank is the absorbance of the control at 30 min, and Abs sample is the absorbance of the essential oil at 30 min. The IC_{50} values of specimens were calculated via linear regression analysis and experiments were carried out in triplicate.

Anti-microbial Activity

The anti-microbial activity of the essential oil, and extracts of *F. stellata* was evaluated via measuring the growth inhibitory zones against four Gram-negative bacteria, three Gram-positive, and one fungal by disc diffusion method [21]. The Gram-negative bacteria included *Escherichia coli* (PTCC 1330), *Enterococcus faecalis* (PTCC 1239), *Salmonella typhi* (PTCC 1230), *Pseudomonas aeruginosa* (PTCC 1047), and Gram-Positive bacteria included *Staphylococcus epidermidis* (PTCC 1435), *Staphylococcus aureus* (PTCC 1337), and *Listeria. monocytogenes* (PTCC 1163) The fungi was *Candida albicans* (PTCC 5027*).* The disc diffusion method was used to evaluate the antimicrobial activity [26]. All bacterial strains were provided from the Pasteur Institute of Iran. The microorganisms from 0.5 McFarland were cultured on Mueller-Hinton agar and the discs (6 mm) were soaked with 25 µL of essential oils at two concentrations in DMSO (100%, and 50% w/v). The bacterial cultures were incubated at room temperature for 30 min and then at 37 °C/24 h. Tetracycline, amikacin, and nystatin were used as the positive controls and, DMSO 1%was used as the negative control. Antibacterial activities of the essential oil and extracts were evaluated by measuring the zone of inhibition around discs plus discs' diameter. All test assays were carried out in triplicate to ensure reproducibility and the results were offered as mean.

Determination of the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC)

The minimal inhibitory concentration (MIC) of the essential oil was obtained using the Mueller-

Hinton broth (MHB) broth micro-dilution method in 96-well microplates. The stock solutions of essential oil and extracts were prepared in DMSO at the final concentration of 20 mg/mL, and then was diluted to 100 µ/mL using agar. The first well was liquated with 50 µL of MHB and 50 µL of essential oils (5% DMSO). Mueller-Hinton broth (100 µL) with 5% DMSO was added to the 9th well (growth control), and 100 µL of MHB was added to the 10thwell (sterility control) as well. A serial 2-fold dilution of the volatile oil was implemented by moving 50 µL of the suspension to the following wells up till the 8th well [27]. For attaining the size of the final inoculum of 5×10^5 CFU per mL based on Wiegand protocol, 0.5 McFarland broth inoculum was diluted in the ratio of 1:100 and added to the wells [28]. The plate was sealed and incubated at 37 °C for 48 h, and the MIC value was considered as the absence of any visible growth of bacteria (clear suspension) [29]. To assess the minimal bactericidal concentration (MBC), a sample from the wells without bacterial growth was cultured in Petri plates with Mueller Hinton Agar (MHA) and stored at 37 °C for 24 h. The MBC value was assigned as the lowest essential oil concentrations that show no bacterial growth [30]. Each experiment was performed in triplicate.

MTT assay

The cytotoxic activity of all extracts was evaluated using the [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) assay as described by Abuali et al. [31]. The human breast cancer cell lines (MCF-7 cells) were obtained from the Pasture Institute of Iran, Tehran, Iran. RPMI 1640 culture medium, with suitable additives containing antibiotics and 10% fetal bovine serum (FBS), was used. The cell line was incubated in an air atmosphere enriched with 5% CO2 at 37 °C. For the MTT assay, the cell lines were cultured and then 100 µL of cell solution at the density of 5×10^3 cells/mL was plated in 96-well plates and incubated for 24 h. Then, the culture media was substituted with fresh media (200 µL), and the cells were treated with different concentrations (10, 50, 100, 200, 300, 400, 500 µg/mL) of the extracts. The stock solutions of extracts were prepared in DMSO at the final concentration of 20 mg/mL, and then was diluted to 500 µ/mL using RPMI1640. After 24 h of incubation, each well received 20 µL of MTT solution (MTT; 5 mg/mL in phosphate-buffered saline) and the plates were incubated at 37 °C in 5% CO2 atmosphere for 4 h. The final concentration of MTT in each well was 0.45 mg/mL. Finally, MTT reduction was measured at 570 nm by multi well ELISA plate reader. The cell viability (%) was attained according to the following Equation:

$$\text{Cell viability (\%)} = \left(\frac{\text{OD sample}}{\text{OD control}}\right) \times 100$$

Where OD sample is the absorbance of the sample and OD control is the absorbance of the negative control. The tests were performed in triplicate, and doxorubicin and DMSO were considered as the positive and negative controls respectively.

Ellman′s assay

The effect of the extracts on acetylcholinesterase (AChE) activity was determined by the microtitre plate assay using acetylthiocholine as a substrate based on the colorimetric method of Ellman et al. [32]. Galantamine and DMSO 10 % were used as positive and negative controls, respectively. In 96-well plates, 200 µL of 0.1 mM sodium phosphate buffer, 40 µL of plant extract (500 µg/mL), 40 µL DTNB (5,5′-Dithiobis (2-nitrobenzoic acid) and 4 µL of AChE solution (0.22 U/mL) were mixed and were incubated for 15 min at room temperature. After that, the acetylcholine iodide (100 µL of 0.05 mM water solution) was added and the AChE inhibition was measured by an ELISA plate reader for 3 min at 412 nm. The concentration of the extracts which inhibited 50% of AChE activity (IC_{50}) was calculated.

Statistical analysis

All experiments were undertaken in triplicates, and the data were presented as mean ± standard deviation (SD). The statistical analysis of results was obtained by GraphPad Prism (Version 8.4.3) and Excel software. Two-way analysis of variance (ANOVA) followed by Tukey's test was used to detect the significance among the groups. Significance was carried out at $p \leq 0.05$.

Results and Discussion

The fruits of *F. stellata* yielded 2.1% of a yellowish essential oil with a pleasant odor and benzaldehyde-like smell. The composition of the essential oil was analyzed by GC-MS and the result is presented in Table 1.

Table 1. Main chemical composition of volatile oil of *Ferulago stellata* fruit

NO.	Name	Percentage	Reported KI	Calculated KI
1	Alpha-pinene	1.92	932	905
2	1,2,4-Trimethylbenzene	1.98	975	946
3	1,2,3-Trimethylbenzene	0.39	1000	967
4	D-Limonene	0.61	1024	978
5	6 – Camphenone	2.84	1095	1021
6	Verbenol	0.97	1137	1063
7	p-Cymen-8-OL	1.10	1179	1087
8	Myrtenal	2.7	1195	1153
9	Benzenepropanol	3.75	1261	1234
10	**2,4,6-Trimethyl benzaldehyde**	**56.05**	1334	1316
11	Beta-caryophyllene	0.91	1408	1397
12	Butanoic acid, 2-methyl-, 2-phenylethyl ester	0.52	1466	1455
13	Spathulenol	1.60	1578	1510
14	Caryophyllene oxide	1.47	1583	1534
	Total	**76.81**		
	Non-terpene	**62.69**		
	Terpene	**14.12**		

KI: Kovats index

Fourteen components were identified representing approximately 76.81 % of the total composition, with 2,4,5-trimethyl benzaldehyde (56.05 %) to be predominating.

The volatile oil of *F. stellata* is mostly composed of oxygenated hydrocarbon (non-terpenoid aromatic) with a small percentage of monoterpenes and sesquiterpenes.

The results are in line with to the relevant studies about essential oils obtained from the aerial part of *F. stellata* with some differences in component proportions [33].

The antioxidant activity of the essential oil of the fruits was evaluated using a spectrophotometer and exhibited a high radical scavenging effect in the DPPH method (IC_{50}=6.05±0.77 µg/mL) comparing to quercetin (IC_{50}=3± 0.00 µg/mL). The high antioxidant activity of essential oils may be linked to the presence of active compounds especially the main constituents in their composition although the presence of other compounds in small quantities could be effective [34,35].

The cytotoxic activity of different extracts of the fruits were assessed on MCF-7 using MTT assay. The IC_{50} values are presented in Table 2. All extracts (regarding IC_{50}) showed low degrees of cytotoxicity against the MCF-7 cell line. Although, p-value of n-hexane extract compared to negative control and other extracts were P <0.001 and p< 0.01, respectively. The chloroform extract revealed no significant activity. It is worth mentioning that the cytotoxic activity of all extracts were insignificant compared to the positive control (IC_{50}=0.43±1.30, p>0.05). Based on the literature review, cytotoxic studies of extracts obtained from different Ferulago species showed promising biological activity [36-39]. Besides, some coumarins like felamedin [40], xanthotoxin, oxypeucedanin, oxypeucedanin hydrate, and isoimperatorin [41] exist in many species of *Ferulago* revealed antiproliferative activity against several tumor cells lines. Karimian et al. investigated anticancer activity *F. angulata* leaves hexane extract against MCF-7 cells and found cytotoxic effects with IC_{50} value of 5.3±0.82 µg/mL [36]. They suggested that the extract suppressed the proliferation of cells by cell cycle arrest and the induction of apoptosis though intrinsic pathway. In another study, antiproliferative effects of lyophilized extract obtained from the aerial parts and the roots of *F. mughlea* demonstrated potent inhibitory effects on cell proliferation [37]. The aerial part inhibited the proliferation of colorectal carcinoma cells (SW-480) at the 48th hour with an IC_{50} value of 0.119 mg/mL.

Table 2. Cytotoxicity and acetylcholinesterase inhibitory activity of *Ferulago stellata* extracts

	IC_{50} (µg/mL)	
Sample	**MTT assay**	**Elman's assay**
n-Hexane	181 ± 28.90^{a,b}	267.51 ± 3.27
Chloroform	> 500	284.35 ± 2.11
Ethyl acetate	392 ± 6.70^{a}	301.14 ± 3.70
Methanol	293 ± 13.40^{a}	234 .23 ± 4.21
Doxorubicin	0.43 ± 1.30	ND
Galantamine	ND*	6.27 ± 2.20

MCF-7 cell line; *: not determined; a: p value<0.001 (all extracts (except chloroform) compared to negative control; b: p value<0.01 (n-hexane extract compared to other extracts)

The results of IC_{50} values for AChE inhibition of all extracts are represented in Table 2. However, the IC_{50} value of the methanol extract (234.23 ± 4.21 µg/mL) in comparison with other extract was low; p-value of all extracts in comparison with galantamine (as positive control) was >0.05. According to our review of the literature, there are few studies on the cholinesterase inhibitory activity of *Ferulago* spp. For instance, the CH_2Cl_2 extracts of the roots of *F. isaurica*, *F. syriaca* [42], *F. blancheana*, *F. pachyloba*, *F. bracteata*, and *F. trachycarpa* [43] significantly inhibited butyrylcholinesterase (BuChE) and AChE (especially *F. bracteata* against BuChE 99.78% and *F. blancheana* against AChE 55.67%) at 20 µg/mL. In another study, the CH_2Cl_2 extract of *F. cassia* root has been detected to show significant BuChE (96.56%), and acetylcholinesterase (AChE) (53.24%) inhibition at 20 µg/mL [9]. To the best of our knowledge, this is the first report about the anticholinesterase activity of extracts from these species.

In herbal medicine, essential oils are considered to be effective anti-microbial agents which can affect microorganisms in a variety of ways, including destroying bacterial cell walls, stopping the translation of specific regulatory gene products, and interfering with bacterial enzyme performance [44]. The antimicrobial activity of the extracts and essential oils against selected microorganisms was evaluated by the absence or presence of inhibition zones, zone diameters, and MIC values, and the results were compared with the standard antimicrobial drugs such as tetracycline and amikacin. The results of the antibacterial effect of the extracts and two concentrations of the essential oil of *F. stellata* fruits on the growth of various strains by the agar disk diffusion method are presented in Table 3.

As shown in Table 3, the amount of diameter of inhibition zone (DIZ) of essential oil, compared to extracts and negative control (DIZ=0 mm) is high. Furthermore, although essential oil significantly inhibits the growth of microbial species in comparison with extracts and negative control ($p<0.001$), in terms of positive control, p-value >0.05 was acquired. It is worth mentioning that the amount of diameter of the inhibition zone of essential oil (100%) against *E. coli* (24±1) mm and *S. typhi* (22 ± 1.30) mm, is more than the amount of the positive control (amikacin).

Table 3. Antibacterial effect of the extracts and essential oil of *Ferulago stellate* fruits on the growth of various strains by agar disk diffusion method

Microorganisms	Diameter of inhibition zone (mm)								
	Essential oil (100%)	**Essential oil (50%)**	***n*-Hexane**	**Chloroform**	**Ethyl acetate**	**Methanol**	**Tetracycline**	**Amikacin**	**Nystatin**
Escherichia coli (PTCC 1330)	24±1.00*	21±0.51	8 ±0.22	8±0.33	10±0.12	15±0.30	NT	21±0.71	NT
Enterococcus faecalis (PTCC 1239)	19±0.50*	16±0.63	10±0.11	8±0.42	10±0.22	13±0.41	NT	25±0.64	NT
Salmonella typhi (PTCC 1230)	22±1.30*	17±0.42	11±0.72	10±0.54	9±0.60	14±0.32	NT	20±1.42	NT
Pseudomonas aeruginosa (PTCC 1047)	22±0.90*	18±0.50	13±0.31	7±0.30	11±0.21	15±0.11	NT	30±1.30	NT
Staphylococcus epidermidis (PTCC 1435)	30±0.80*	24±1.20	11±0.52	10±0.41	12±0.11	16±0.31	36±0.90	NT	NT
Staphylococcus aureus (PTCC 1337)	37±1.10	27±1.00	10±1.00	9±0.62	11±0.52	17±0.30	41±0.70	NT	NT
Listeria monocytogenes (PTCC 1163)	32±1.32	28±0.60	12±0.51	12±0.54	14±0.80	15±0.52	40±0.81	NT	NT
Candida albicans (PTCC 5027)	23±0.81	21±0.52	10±0.61	8±0.34	10±0.41	14±0.63	NT	NT	30±1.11

NT: not tested, *Essential oil (100%) compared to other extracts inhibited microorganism significantly ($p<0.001$). Samples did not demonstrate significant activity compared to the positive controls ($p>0.05$).

However, among the extracts, the amount of DIZ of methanol extract is high (also MeOH extract decreased the growth of above-mentioned microorganisms significantly compared to other extracts and negative control, $p<0.01$), its p-value compared to positive control is non-significant ($p>0.05$). In general, the volatile oil showed powerful antimicrobial activity against both Gram-negative and Gram-positive bacteria. The result of the MIC/MBC assay is presented in Table 4.

In terms of MIC and MBC, the highest activity was achieved against *E. coli* (MIC=0.78 µg/mL, MBC=1.56 µg/mL), *P. aeruginosa* (MIC = 0.78 µg/mL, MBC=1.56 µg/mL), *L. monocytogenes* (MIC=0.78 µg/mL, MBC=1.56 µg/mL) and *Candida albicans* (MIC=0.78 µg/mL, MBC=1.56 µg/mL). The result showed that the essential oil was less active against *E. faecalis* (MIC=3.12 µg/mL, MBC=6.25 µg/mL) and was verified by the lowest zone of inhibition *(*19 ± 0.50 mm*)* obtained against *E. faecalis.* The extracts of the fruit represented lower activity in comparison to the essential oil and the order of its activity was as fallow; methanol>ethyl acetate>n-hexane>chloroform. There are numerous studies regarding the antimicrobial activity of the essential oil and extracts of *Ferulago* spp. [39,45-48]. Golfakhrabadi et al. investigated antimicrobial activity of the extracts, and essential oil of *F. carduchorum* against *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* and they found that the essential oils showed more potent antimicrobial activity than its extract and fractions [39]. In another study, they evaluated the biological activity of four fractions and crude extract of aerial parts of *F. carduchorum* in two vegetative stages (flower and fruit). The results demonstrated that the antimicrobial activity during the flowering was higher than the fruit season, and all the fractions exhibited moderate activity against Gram-positive bacteria and lower active against Gram-negative bacteria [45]. Shahbazi and Shavisi found that the essential oil of the aerial parts of *F. bernardii* presented antibacterial effects on the growth of all tested bacteria which was significantly higher than the nonpolar and polar sub-fractions of the methanolic extract [47]. Darderafshi et al. reported that the essential oil of *F. angulata* at high concentrations could prevent microbial contaminations and might be used in dairy products to extend the shelf-life of foods (such as cheese) as a substitution to chemical preservatives [49]. Those reported studies are in favor of the potent antimicrobial activity of different *Ferulago* spp., but few reports indicate no significant activity against tested microorganisms [50,51]. Bostanlik et al. found that the extracts of *F. mughlae*, and *F. sandrasica* had no activity against *P. aeruginosa*, *E. coli*, and *B. subtilis* [50]. Azarbani et al. found that the methanol and water extracts of leaf, flower, and stem of *F. angulata* showed no significant activity against *E. coli*, *S. aureus*, *Bacillus cereus and Klebsiella pneumonia.*

Table 4. The antimicrobial activity of *Ferulago stellata* (extracts and essential oil) indicated as minimum inhibitory/bactericidal concentrations-MIC/MBC (µg/mL)

Microorganisms	Essential oil (µg/mL)		Extract (MIC (µg/mL)			
	MIC	MBC	*n*-Hexane MIC	Chloroform MIC	Ethyl acetate MIC	Methanol MIC
Escherichia coli (PTCC 1330)	0.78	1.56	*12.5*	25	6.25	6.25
Enterococcus faecalis (PTCC 1239)	3.12	6.25	25	25	12.5	12.5
Salmonella typhi (PTCC 1230)	1.56	3.12	25	25	12.5	6.25
Pseudomonas aeruginosa (PTCC 1047)	0.78	1.56	12.5	25	12.5	6.25
Staphylococcus epidermidis (PTCC 1435)	1.56	3.12	12.5	25	12.5	6.25
Staphylococcus aureus (PTCC 1337)	1.56	3.12	12.5	25	12.5	6.25
Listeria monocytogenes (PTCC 1163)	0.78	1.56	12.5	12.5	12.5	6.25
Candida albicans (PTCC 5027)	0.78	1.56	25	25	12.5	12.5

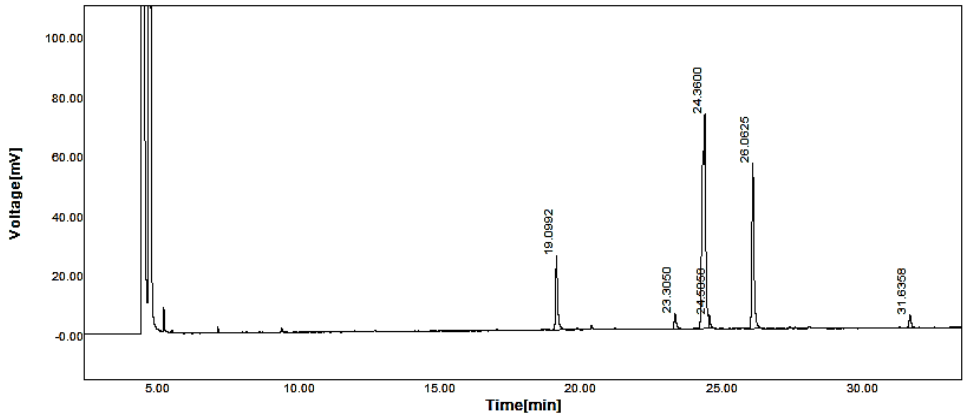


Figure 1. The chromatogram of fatty acid methyl esters attained from the *n*-hexane extract of *Ferulago stellata fruits*

Table 5. Fatty acid composition, percentage, retention time, and the structures of compounds from *Ferulago stellata* fruits

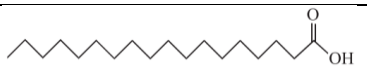
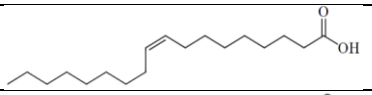
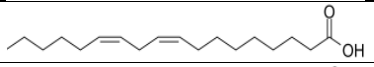
Composition	RT (min)	Percentage	Structure
Palmitic acid (C16:0)	19.09	11.23	
Stearic acid (C18:0)	23.30	2.31	
Oleic acid (C18:1n9c)	24.36	56.14	
Linoleic acid (C18:2n6c)	26.06	26.61	
Docosanoic acid (C22:0)	31.63	2.08	

Figure 1 is a schematic view of the fatty acid profile of *F. stellata* fruits determined by capillary-column GC. It shows five peaks identified during nineteen to thirty-two minutes. The highest peak corresponded to oleic acid and was displayed at the retention time 24.36 min; the lowest one belonged to docosanoic acid at the retention time of 31.64 min. The name of five fatty acid compositions, oil content (%), retention time, and their structures are presented in Table 5. Table 5 illustrates palmitic acid (C16:0), stearic acid (C18:0), and docosanoic acid (C22:0) are saturated fatty acids identified from *F. stellata* fruits. The order of amount was as follows: palmitic acid (11.23)>stearic acid (2.31)> docosanoic acid (2.08). The only monounsaturated fatty acid and the highest fatty acid composition of the fruits was oleic acid (56.14 %). Oleic acid, the main fatty acid of olive oil, is an effective cholesterol-lowering agent potentially beneficial in the prevention and treatment of inflammatory, cardiovascular, and autoimmune diseases [13,52]. Ghafoor et al. (2019) found that oleic acid was the highest fatty acid composition in fruits of *F. pachyloba* (68.82%), *F. pauciradiata* (67.15%), *F. syraea* (67.15%), and *F. syriaca* (66.16%) [53]. Linoleic acid (C 18:2, 26.61%) was the only polyunsaturated fatty acid found in the fatty acid composition of the fruits. The high content of oleic and linoleic acids in the fatty acid profile of this species was considerable. Erdemoğlu et al. (2008) found that the fatty acid profile from fruits oil of *F. trachycarpa* from Konya and Balıkesir provinces (Turkey) contained 73.6% and 68.1% oleic, and 18.0% and 23.0% linoleic acids, respectively [52]. The differences in the quantity of oleic and linoleic acids from fruits of *F. stellata* and those reported here might be probably due to different geographical features, plant nutrition, and light, etc. [53]. The analysis of the fatty acid displayed that the quantities of saturated fatty acids and unsaturated fatty acids were 15.62%, 82.75, respectively that indicates a high unsaturated/saturated ratio.

Conclusion

This study focused on the chemical composition, antioxidant and antimicrobial activity of *F. stellata* essential oil for the first time. The result of the fatty acid profile showed that the fruits are considerable sources of unsaturated fatty acids. The present study indicated that the volatile oil of *F. stellata* is a source of antioxidant, antimicrobial, and healthy fatty acid compounds and could be used in the pharmaceutical, cosmetic, and food industries.

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Author contributions

Yahya Rahimpour performed the experiments and prepared the manuscript; Abbas Delazar and Parina Asgharian designed and supervised the study; Samad Nejad Ebrahimi analyzed the data; Mahsa Sabernavaei performed and evaluated some experiments. All authors read and approved the final manuscript.

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

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; DMSO: dimethyl sulfoxide; FBS: fetal bovine serum; GC/MS: gas chromatography-mass spectrometry; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; RI: retention indices; TPC: total phenolics compounds; TFC: total flavonoids content; AChE: acetylcholinesterase