Journal of Essential Oil Research

ISSN: 1041-2905 (Print) 2163-8152 (Online) Journal homepage: http://www.tandfonline.com/loi/tjeo20

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To cite this article: S. Tavakoli, N. Yassa, M. R. Delnavazi, M. Akhbari, A. Hadjiakhoondi, H. Hajimehdipoor, F. Khalighi-Sigaroodi & R. Hajiaghaee (2017) Chemical composition and biological activities of the essential oils from different parts of Ferulago trifida Boiss, Journal of Essential Oil Research, 29:5, 407-419, DOI: 10.1080/10412905.2017.1313178

To link to this article: http://dx.doi.org/10.1080/10412905.2017.1313178

Published online: 17 Apr 2017.

JOURNAL OF ESSENTIAL OIL RESEARCH, 2017
VOL. 29, NO. 5, 407–419
https://doi.org/10.1080/10412905.2017.1313178

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Chemical composition and biological activities of the essential oils from different parts of *Ferulago trifida* Boiss

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ABSTRACT

As a result of GC and GC-MS analysis of the essential oils from different parts of *Ferulago trifida* Boiss., a total of thirty-three compounds were identified in the flowers (Fl), stems (S), leaves (L) and fruits (Fr) essential oils, of which (E)-β-ocimene (Fl: 37.3, S: 20.7, L: 25.7, Fr: 30.5%), α-pinene (Fl: 16.3, S: 22.6, L: 19.6, Fr: 18.0%) and bornyl acetate (Fl: 9.4, S: 8.5, L: 16.7, Fr: 11.0%) were main compounds. Among the thrity-eight compounds identified in the roots essential oil, suberosin (20.7%), β-barbatene (6.6%) and cuparene (6.1%) were main compounds. All essential oils exhibited weak DPPH free radical scavenging activity (IC_{50}: 95–120 μg ml^{-1}). Among the tested samples flowers, fruits and roots essential oils by 72.1, 78.7 and 74.3% inhibition of Ach Enzyme, respectively, exhibited considerable AChE inhibitory effects in Elman method. They had moderate cytotoxic activity (IC_{50}: 15–55 μg ml^{-1}) on cancerous cell lines (MCF-7, A-549 and HT-29) in MTT assay.

ARTICLE HISTORY
Received 7 March 2016
Accepted 6 March 2017

KEYWORDS
Ferulago trifida Boiss; essential oil; antimicrobial effect; antioxidant; AChE inhibitory effect; MTT assay

Introduction

Essential oils extracted from aromatic plants are known since ancient times for their fragrance, antiseptic, food preservative and medicinal properties (1). In medical and medicinal sciences, essential oils have been considered as a source of biologically active compounds, as well as for their skin penetration enhancer properties (in trans dermal drug delivery systems) and their proposed therapeutic values in massage and aromatherapy (2).

The genus *Ferulago* W. D. Koch from Apiaceae family consists of fourty-six species distributed in Europe, Africa and Asia (3). The plants belonging to this genus are perennial aromatic species, of which some are used as spice or medicinal plant (4–6). In Turkey, the aerial parts of some *Ferulago* species are used as flavour, sedative, tonic, digestive, immunostimulant, anti-bronchitis and wormicidal. The roots of some *Ferulago* species are also used as aphrodisiac and for the treatment of cancers and skin diseases in Turkish folk medicine (4,5). In north of Iraq, it is reported that the fresh or dried leaves of *F. abbreviata* are used by indigenous people as foot deodorant (6). The aromatic aerial parts of *F. angulata* are also used in the west of Iran as flavour and food preservative (7).

So far, a number of *Ferulago* species have been investigated for phytochemical constituents of their flowers, stems, leaves, fruits and roots essential oils (8–20). The main compounds (compounds with relative amount upper than 5.0%) identified in the essential oils of these species have been summarized in Table 1. Some *Ferulago* species have also been reported for their antibacterial, antifungal and antioxidant activities (9–11,13,16,19,20). Moreover, antiamnesic, antioxidants, anxiolytic and antidepressant-like effects of the essential oil of *F. angulata* aerial parts have been shown during previous biological studies (21,22).

Ferulago trifida Boiss. is one of the nine *Ferulago* species represented in flora of Iran. This species is a perennial plant with up to 1.5 m in height which grows as an endemic plant in northwest of Iran (23). Regarding to the importance of *Ferulago* species as aromatic plants with various medicinal potentials, the present study was designed to investigate chemical composition, antioxidant,

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Table 1. The main compounds of 22 *Ferulago* species essential oils analysed in previous studies.

Species (Ref.)	Part	Method	Yield (%)	Main compounds (%)
F. angulate (8)	Flowers	HD[a]	0.66	α-phellandrene (27.2), β-phellandrene (16.6), α-pinene (12.2) and *p*-cymene (10.3)
	Stems	HD	0.54	α-phellandrene (18.1), β-phellandrene (15.8), α-pinene (21.2) and *p*-cymene (17.7)
	Leaves	HD	0.43	α-phellandrene (20.7), β-phellandrene (16.2), α-pinene (16.8) and *p*-cymene (14.6)
F. compestris (9, 10)	Flowers	HD	0.4–0.7	α-pinene (8.3–16.2), myrcene (9.5–18.7) and γ-terpinene (8.3–32.9)
	Leaves	HD	0.05–0.06	α-humulene (0.6–5.1), spathulenol (2.9–5.5), caryophyllene oxide (4.0–5.9), humulene epoxide II (2.2–5.4) and α-cadinol (0.4–5.9)
	Fruits	HD	5.7–7.1	Myrcene (33.4–39.7), α-pinene (22.7–23.0), γ-terpinene (8.1–10.9) and 2,3,6-trimethylbenzaldehyde (8.6–9.0)
	Roots	HD	0.3–0.8	α-pinene (58.3–75.0) and 2,3,6-trimethylbenzaldehyde (14.8–27.9)
F. contracta (11)	Flowers	HD	1.3	Germacrene D (9.8), α-pinene (5.3) and bicyclogermacrene (5.1)
	Stems	HD	0.4	(E)-β-ocimene (11.3), *p*-cymene (9.4), spathulenol (7.4), germacrene D (6.3), β-eudesmol (5.9) and linalool (5.2)
	Leaves	HD	0.5	β-eudesmol (24.5), spathulenol (16.2), citronellol (11.9) and linalool (6.8)
F. isaurica (12)	Fruits	HD	12.0	α-pinene (31.5), limonene (24.2) and myrcene (16.9)
	Roots	HD	0.7	Terpinolene (42.1) and myrcene (28)
F. syriaca (12)	Fruits	HD	4.8	Myrcene (15.3), 4,6-guaiadiene (10.7), 2,3,6-trimethylbenzaldehyde (8.8) and cubenol (8.8)
	Roots	HD	1.1	Bornyl acetate (69.4) and terpinolene (12.5)
F. Macedonia (13)	Flower	HD	0.02	α-pinene (43.1) and sabinene (26.7)
F. tirkeana (14)	Fruit	HD	4.1	Ferulagone (63.5), germacrene D (14.0) and α-pinene (9.0)
	Fruit	MD[b]	–	Ferulagone (56.3), germacrene D (12.5) and α-pinene (10.3)
F. asparagifolia (15)	Fruits	HD	7.0	2,3,6-trimethyl benzaldehyde (38.9) and myrcene (18.2)
F. longistylis (16)	Fruit	HD	6.4	2,3,6-trimethylbenzaldehyde (29.4), α-pinene (16.7), (Z)-β-ocimene (15.9), sabinene (6.2) and myrcene (5.7)
F. macrocarpa (17)	Fruit	HD	0.8	Bornyl acetate (40.8%), 2,3,6-trimethyl benzaldehyde (7.2%), δ-selinene (5.5%), 1,10-di-epi-cubenol (5.1%)
F. asparagifolia (18)	Fruits	MD	–	2,3,6-trimethylbenzaldehyde (42.0) and α-pinene (11.4)
F. aucheri (18)	Fruits	MD	–	α-pinene (35.9), humulene epoxide-II (7.3) and *trans*-verbenol (6.4)
F. confuse (18, 20)	Fruits	MD	–	2,5-dimethoxy-*p*-cymene (63.4) and *p*-cymene (24.4)
	Fruits	HD	2.4	*Cis*-chrysanthenyl acetate (37.7) and α-pinene (36.7)
F. galbanifera (18)	Fruits	MD	–	*Trans*-chrysanthenyl acetate (17.2), *p*-cymene (11.9), α-phellandrene (10.9), limonene (10.3), β-phellandrene (7.8), β-caryophyllene (7.8) and linalool (5.0)
F. humilis (18)	Fruits	MD	–	(Z)-β-ocimene (31.9), limonene (31.4), *p*-cymene (7.0) and α-pinene (6.1)
F. idaea(18)	Fruits	MD	–	*p*-cymene (18.4), α-pinene (16.1), 2,3,6-trimethylbenzaldehyde (14.1), carvacrol methyl ether (13.4), 2,5-dimethoxy-*p*-cymene (13.2) and trans-chrysanthenyl acetate (8.8)
F. macrosciadia (18)	Fruits	MD	–	Carvacrol methyl ether (78.1) and *p*-cymene (19.4)
F. mughlae (18)	Fruits	MD	–	α-pinene (25.4), cubenol (12.7) and α-phellandrene (10.9)
F. sandrasica (18)	Fruits	MD	–	α-pinene (40.8), α-humulene (8.1) and germacrene D (5.8)
F. sandrasica (19)	Leaves	HD	–	Ocimene (30.5), careen-6_3(27.4) and α-pinene(17.8)
F. silaifolia (18)	Fruits	MD	–	*Trans*-chrysanthenyl acetate (83.5) and α-pinene (5.6)
F. sylvatica (18)	Fruits	MD	–	*p*-cymene (45.8)
F. trachycarpa (8)	Fruits	MD	–	γ-terpinene (27.8), *p*-cymene (21.6) and myrcene (19.9)

[a]Hydrodistillation.
[b]Micro-distillation.

antimicrobial, acetyl cholin esterase (AChE) inhibitory, general toxicity and cytotoxic activity of the essential oils obtained from the flowers, stems, leaves, fruits and roots of *F. trifida*. This study reports chemical composition and biological activities *F. trifida* essential oils for the first time. To the best of our knowledge this is also the first report on AChE inhibitory, general toxicity and cytotoxic activity of the essential oils from different parts of a plant belonging to the genus *Ferulago*.

Material and methods

Plant material

The flowers, stems, leaves and roots of *F. trifida* Boiss. were collected in July 2014 and the fruits were gathered in August 2014 from around of ovan Lake located in Alamut region, Ghazvin province, Iran. The plant was identified by Botanist, Professor V. Mozaffarian (Research Institute of Forest and Rangelands, Tehran, Iran) and its voucher specimen was deposited under the code of THE-6562 in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Solvents and chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), acetyl thiocholine iodide (ATCI), acetyl cholin esterase enzyme (AChE) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide(MTT) were procured from Sigma–Aldrich chemical Co., Germany. All solvents, anhydrous sodium sulphate, sodium carbonate, tween 80, all microbial culture media, 5,5′-Dithiobis-2-nitrobenzoic acid (DTNB) were obtained from Merck chemical Co., Germany. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were also purchased from GIBCO, USA.

Essential oils

The shade-dried and comminuted plant samples (200 g each) were individually subjected to hydrodistillation for 3 h using a Clevenger apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and kept at 4 °C until analyses.

GC and GC-MS analysis

Essential oils of different parts of *F. trifida* were analysed on a HP-6890 gas chromatograph with a HP-5MS column (30 m × 0.25 mm id, 0.25 μmfilm thickness), equipped with HP-5973 mass detector (Ionization energy: 70 eV) under the following conditions; temperature program: 60 °C (0–3 min), 60–250 °C at the rate of 3 °C/min (3–65 min), injector temperature: 220 °C, detector temperature: 290 °C, injection volume: 1.0 μl, split ratio: 1:90, carrier gas: helium (Flow rate: 1 ml min^{-1}). The Kovats retention indices (KIs) were calculated for all identified compounds using a homologous series of *n*- alkanes injected under the same conditions described to samples. Identification of the compounds were carried out based on computer matching with the Wiley275.L and Wiley7n.L libraries, as well as by comparison of KIs and mass fragmentation patterns with those published for standard compounds (24).

For quantitative analysis, essential oils were also injected to HP-6890 gas chromatograph with a HP-5MS column fitted with FID detector in conditions equal to GC–MS analysis. Experiment was repeated three times for each sample and amounts of compounds (real % area) were expressed as Mean ± SD.

Antibacterial activity

Bacterial strains

Antimicrobial activity of the essential oils were individually assessed against a set of seven bacterial strains, *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10536), *Klebsiella pneumonia*(ATCC 10031), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29737), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Salmonella paratyphi* A (ATCC 5702) and *Proteus vulgaris* (PTCC 1182), as well as tree fungi including two mould, *Aspergillus brasiliensis* (ATCC 1015) and *Aspergillus niger* (ATCC 16404) and one yeast, *Candida albicans* (ATCC 10231), provided from Iranian Research Organization for Science and Technology (IROST).

Disc diffusion assay

Disc diffusion method was applied for the evaluations of antimicrobial activity of essential oils (25). Essential oils were filtered through 0.45 μm Millipore filters for sterilization. 100 μl of suspension containing 10^8 CFU/ml of bacteria, 10^4 spore/ml of mouldand10^6 CFU/ml of yeast were spread on the nutrient agar (NA), potato dextrose (PD) agar and sabouraud dextrose (SD) agar mediums, respectively. The impregnated discs (6 mm in diameter) with 10 μl of the essential oils were placed on the inoculated agar. The diameters of inhibition zones (IZ) (mm) were measured following incubation of all plates at 37 °C (for bacteria) and at 30 °C (for fungi) for 24 h. Gentamicin (10 μg/disc) and rifampin (5 μg/disc) were used as positive controls for bacteria and nystatin (100 I.U./disc) for fungi. Each assay was repeated twice and diameters of inhibition zones were represented as mean.

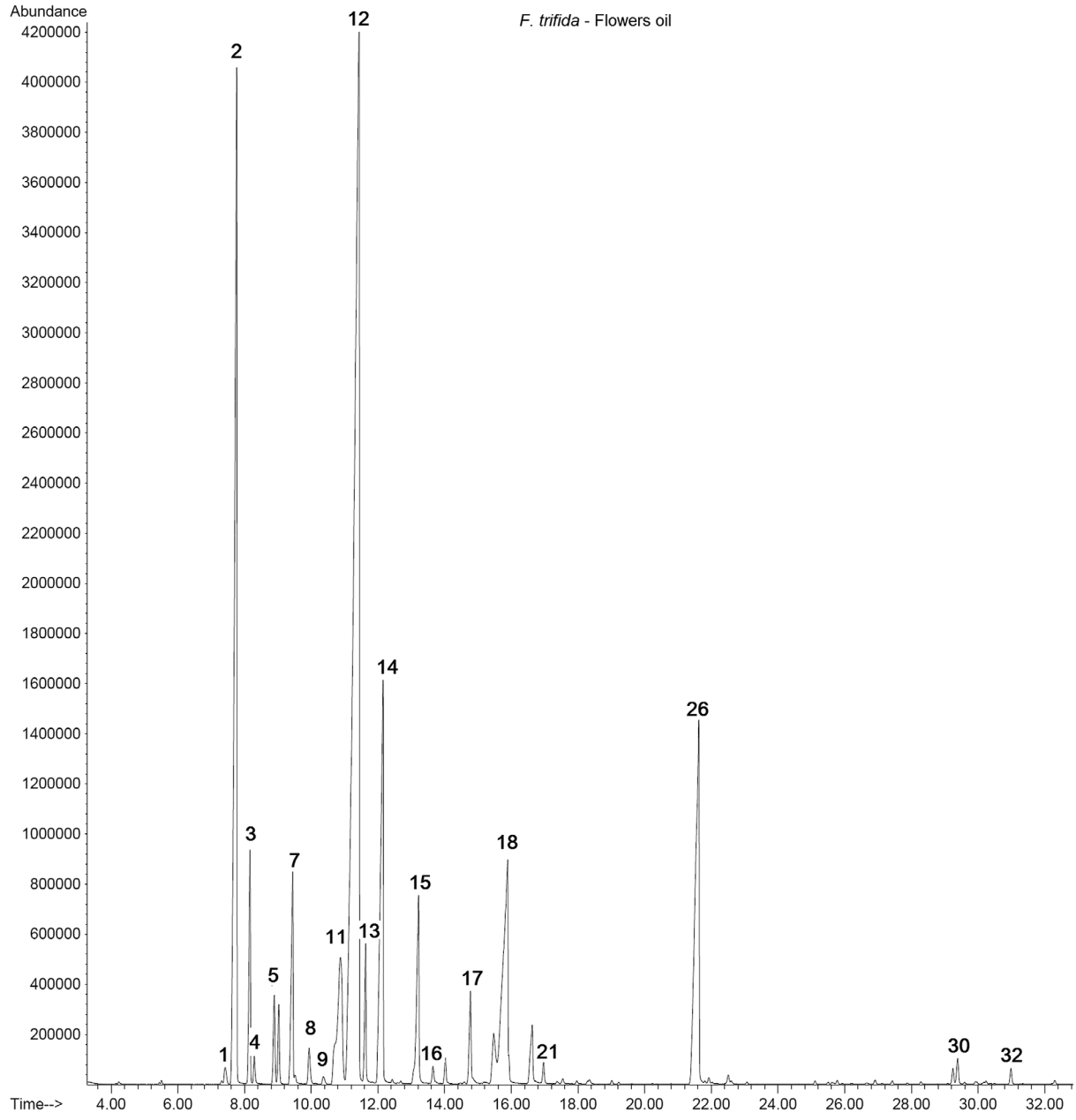

Figure 1. GC–MS chromatogram of the flowers essential oil.

Micro-well dilution assay

Essential oils were subjected to micro-well dilution assay in order to determination of minimal inhibition concentration (MIC) values, for microbial strains which were found susceptible in disc diffusion assay (26). The suspensions of microbial strains were prepared at 0.5 McFarland standard turbidity from their 12 h broth cultures. The serial two-fold dilutions of essential oil samples were made in a concentration range from 7.8 to 500 µg/ml in sterile test tubes containing brain heart infusion (BHI) broth for bacteria and SD broth for fungi strains. 95 µl of the cultures media and 5 µl of the inoculum were dispensed into each well of the 96-well plates. Then, 100 µl from essential oil dilutions was added to wells. A well containing 195 µl of the cultures media and 5 µl of the inoculum without the test sample was used as negative control. Gentamicin and rifampin for bacteria and nystatin for fungi were also used as positive control in same conditions as described to test samples. The content of plates were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 µl samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant essential oils required for inhibiting the growth of microorganisms. All tests were repeated three times.

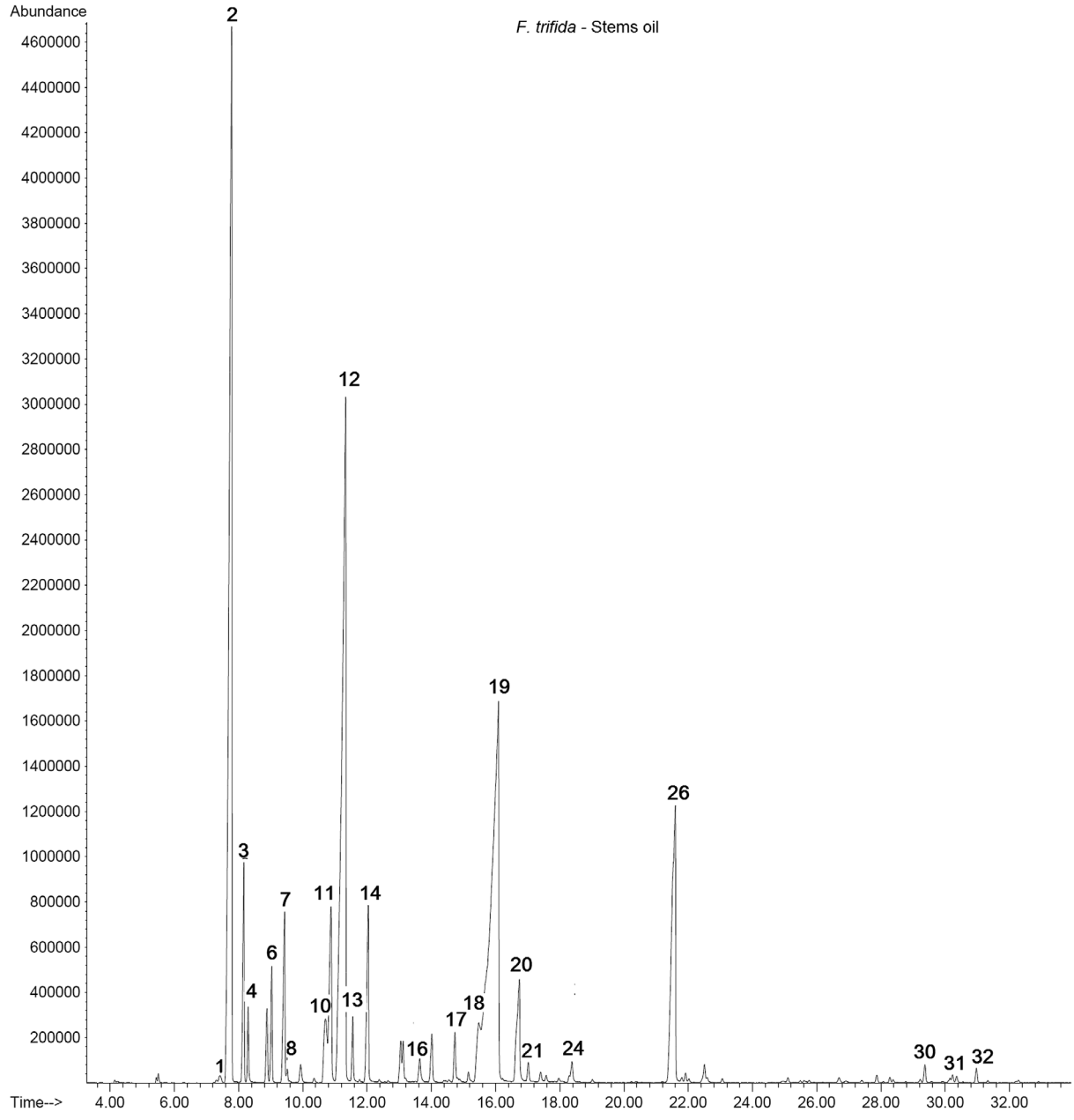

Figure 2. GC–MS chromatogram of the stems essential oil.

DPPH free radical scavenging assay

Antioxidant capacities of the essential oils obtained from different parts of *F. trifida* were determined in DPPH free radical scavenging assay as described by Sarker et al. (27). Briefly, 2.5 ml of DPPH solution (80 µg ml^{-1} in methanol) was added to 2.5 ml of sample solutions prepared in concentrations ranging from 5.0 to 9.5 × 10^{-3} mg ml^{-1}in methanol. After keeping of the solutions in dark for 30 min at 25 °C, UV absorptions were recorded on an Optizen 2120 UV PLUS spectrophotometer (Daejeon, Korean) at 517 nm. BHT (Butylated hydroxyl toluene), a synthetic antioxidant, was used as a positive control. All tests were done in triplicate, and IC_{50} values were reported as Mean ± SD.

Acetylcholinesterase inhibitory assay

AChE inhibitory activities of the samples were determined based on method described by Ellman et al. (1961), with slight modification in a 96-well microplate (28). Briefly, 125 µl of DTNB (3 mM), 25 µl of ATCI (15 mM), 50 µl of phosphate buffer (pH 8) and 25 µl of the essential oil sample solution (3 mg ml^{-1}, in methanol) were added to 96-well plates. The absorbance was recorded at 405 nm in 13 s intervals for 65 s using a TECAN micro plate reader. After that, 25 µL of AChE enzyme (0.22 Uml^{-1}) was added and the absorbance was measured again in 13 s intervals for 104 s. the enzyme activity was calculated from the slope of the line obtained from plotting of the absorbance against time.

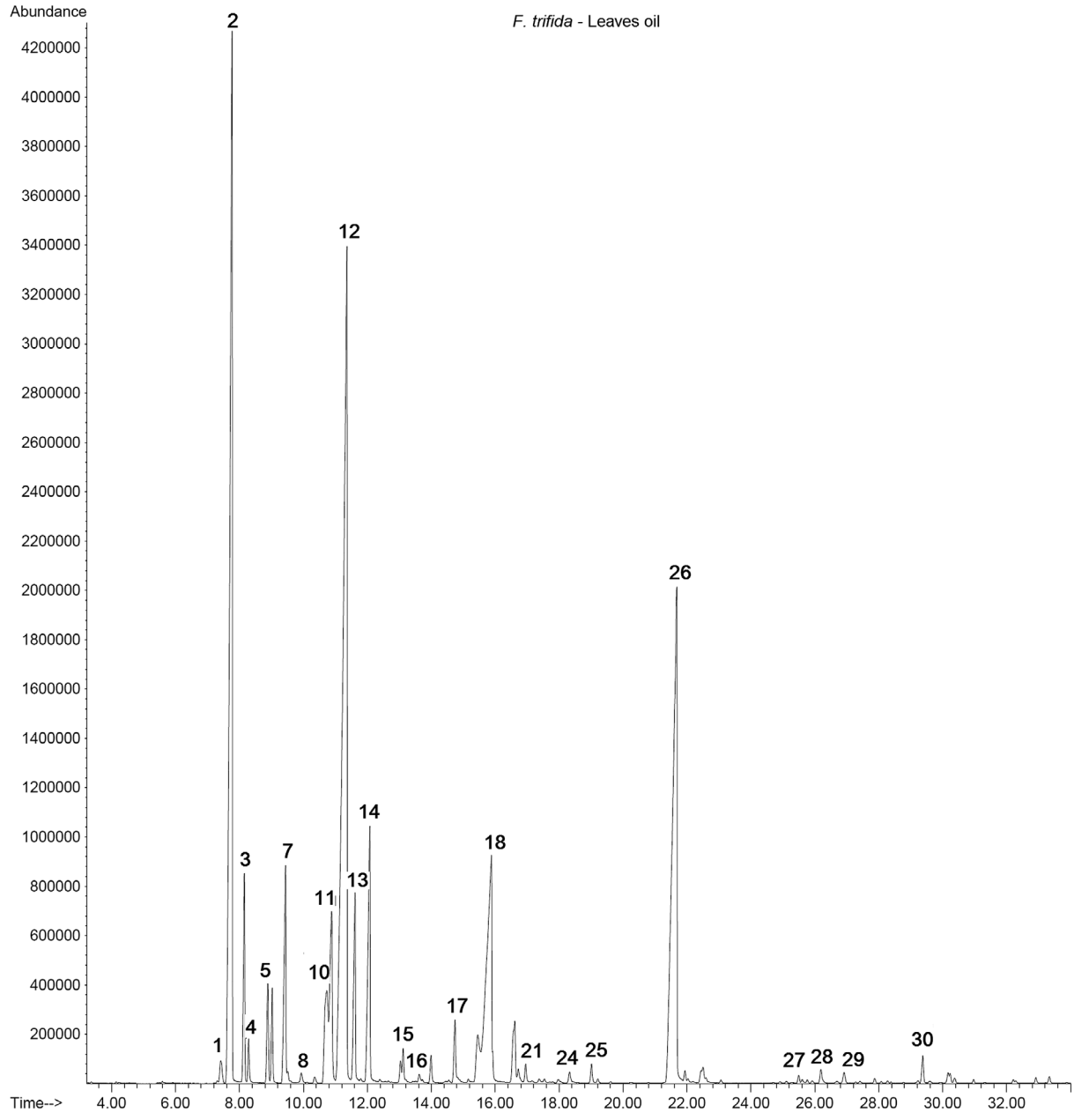

Figure 3. GC–MS chromatogram of the leaves essential oil.

Any increase in the absorbance caused by non-enzymatic hydrolysis of ATCI was corrected by the recorded absorbance before addition of enzyme. Percentage of enzyme inhibition was calculated by comparing the rates for the sample to the blank (using methanol without tested sample). Donepezil, a drug with potent AChE inhibitory activity, was used as the positive control.

Brine shrimp lethality test

General toxicity potentials of the essential oil samples were evaluated using brine shrimp lethality test (BSLT) (29). Artificial sea water was prepared by dissolving of 38 g sea salt in 1.0 L water and adjusted to pH 9 using sodium carbonate. The cysts of *Artemia salina* L. were hatched in sterile artificial seawater under constant aeration for 48 h at 30 °C. Essential oil samples (50 mg) were mixed with DMSO (250 µl) and tween 80 (one drop) and diluted with artificial sea water to get solutions with 1000, 700, 500, 300, 100, 10, 2, 1, 0.5 and 0.25 µg/ml concentrations in a series of tubes containing about twenty active nauplii in each. The tubes were placed in a water bath at 30 °C for 24 h under light, and the surviving nauplii were then counted to obtain the concentration causing 50% lethality (LD_{50} value). Podophyllotoxin, a known cytotoxic natural compound, was applied as positive control. The assay

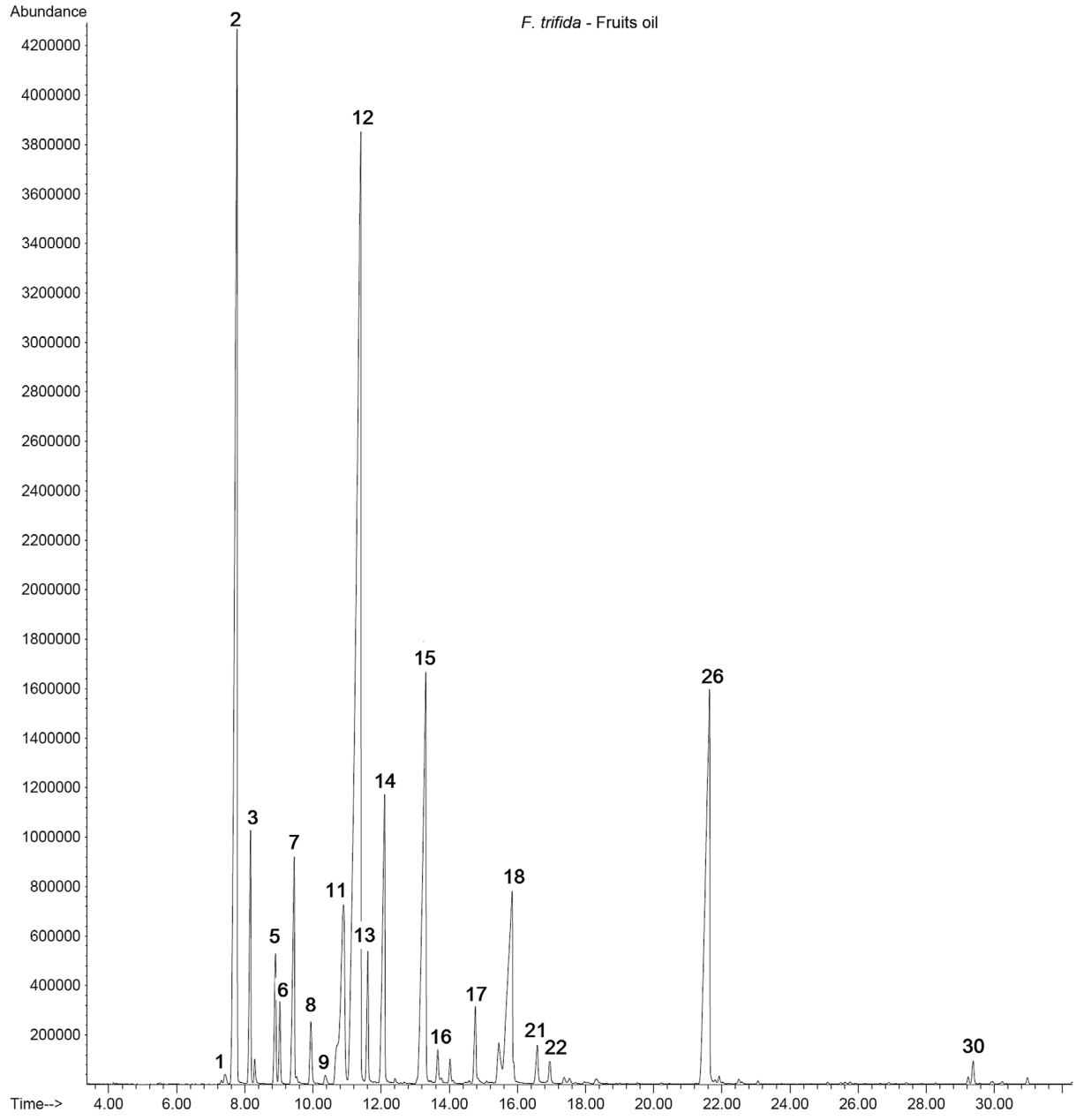

Figure 4. GC–MS chromatogram of the fruits essential oil.

was performed tree times and LD_{50} value was reported as Mean ± SD.

In vitro *cytotoxic activity assay*

Cell lines

Three tumour cell lines, MCF-7 (human breast adenocarcinoma), A-549 (non-small cell line carcinoma) and HT-29 (human colon adenocarcinoma) were obtained from Pasture Institute of Iran, Tehran, Iran. The cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% penicillin–streptomycin in a 5% CO_2 incubator at 37 °C.

MTT assay

In vitro cytotoxic activities of the essential oils obtained from the different parts of *F. trifida* was evaluated in MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide) colourimetric assay (30). Cells were seeded into 96-well plates at a density of 0.5–1.5 × 10^4 cells/well and incubated for 24 h at 37 °C. The medium was then replaced by fresh medium containing different concentrations of essential oils and incubated for 72 h at 37 °C. After that, the medium was replaced by fresh medium containing MTT and incubated for additional 4 h. During this period, MTT is reduced to formazan (purple dye) by living cells. Finally, the precipitated formazan crystals were dissolved in DMSO (200 µl) and absorbance was

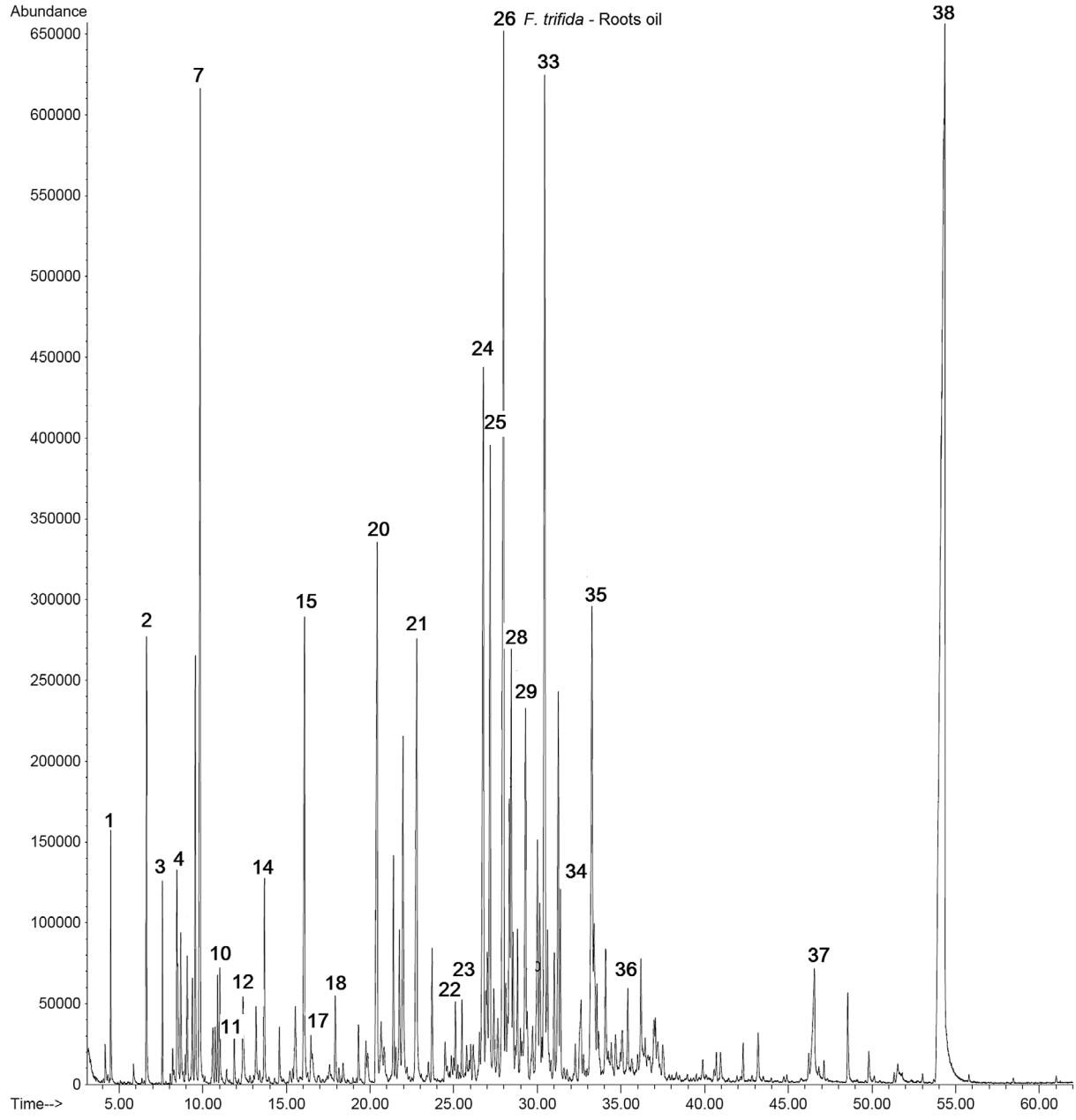

Figure 5. GC–MS chromatogram of the roots essential oil.

recorded at 570 nm, using a TECAN microplate reader. Cytotoxic activity of the essential oils was defined as the concentration causing a 50% reduction in viability of cells relative to the negative control (IC_{50} value). Tamoxifen was also used as positive control.

Results and discussion

Essential oils composition

The hydrodistillation of the flowers (Fl), stems (S), leaves (L), fruits (Fr) and roots (R) of *F. trifida* resulted in pale yellowish essential oils with yields of 1.4, 1.3, 1.6, 1.4 and 0.8% (v/w), respectively. GC and GC–MS analysis the essential oils led to the identification of a total of 33 compounds in the flowers, stems, leaves and fruits essential oils together with thirty-eight compounds in the roots. The chromatograms of the flowers, stems, leaves, fruits and roots essential oils have been presented in Figures 1–5. The results showed that the essential oils of aerial parts were rich in monoterpene hydrocarbons (Fl: 79.1, S: 60.6, L: 68.3, Fr: 79.2%), with (E)-β-ocimene (Fl: 37.3, S: 20.7, L: 25.7, Fr: 30.5%), α-pinene (Fl: 16.3, S: 22.6, L: 19.6, Fr: 18.0%) and bornyl acetate (Fl: 9.4, S: 8.5, L: 16.7, Fr: 11.0%) as main compounds. Furthermore, *cis*-verbenol was identified as one of the main compounds in the flowers (8.6%), leaves (10.4%)

Table 2. Chemical composition of the roots essential oil of *F. trifida*.

No.	Compounds[a]	KI[b]	KI[c]	Real % area
1	Hexanal	803	803	0.6 ± 0.1
2	Heptanal	903	903	1.4 ± 0.1
3	α-pinene	934	934	0.6 ± 0.1
4	o-Ethyl-toluene	964	962	1.7 ± 0.2
5	Pseudocumene	974	971	0.6 ± 0.1
6	2-Amylfuran	987	984	0.5 ± 0.2
7	Octanal	1000	998	4.7 ± 0.5
8	Limonene	1026	1026	0.2 ± 0.1
9	1,8-Cineole	1028	1028	0.4 ± 0.2
10	(E)-β-Ocimene	1046	1046	0.5 ± 0.2
11	γ-Terpinen	1058	1056	0.2 ± 0.1
12	n-Octanol	1064	1062	0.5 ± 0.3
13	2-Nonanone	1090	1089	0.4 ± 0.1
14	n-Nonanal	1103	1103	0.9 ± 0.2
15	cis-verbenol	1133	1134	3.3 ± 0.4
16	(2E)-Nonenal	1163	1165	2.5 ± 0.5
17	Borneol	1174	1175	0.4 ± 0.2
18	n-Decanal	1204	1204	0.4 ± 0.1
19	(2E)-Decenal	1261	1262	0.8 ± 0.2
20	Bornyl acetate	1286	1286	1.0 ± 0.3
21	(2E,4E)-Decadienal	1318	1318	2.9 ± 0.5
22	α-Copaene	1376	1376	0.4 ± 0.1
23	β-bourbonene	1391	1391	0.4 ± 0.1
24	β-Funebrene	1415	1415	4.8 ± 0.6
25	Cis-Thujopsene	1430	1431	0.6 ± 0.1
26	β-Barbatene	1444	1442	6.6 ± 0.5
27	(E)-β-Farnesene	1458	1457	1.7 ± 0.3
28	β-Acoradien	1471	1471	2.1 ± 0.2
29	β-Chamigrene	1478	1479	2.4 ± 0.3
30	γ-Curcumene	1483	1485	0.7 ± 0.2
31	Bicyclogermacrene	1501	1503	1.6 ± 0.3
32	β-Himachalene	1500	1505	1.8 ± 0.6
33	Cuparene	1507	1509	6.1 ± 1.3
34	δ-Cadinene	1521	1523	0.6 ± 0.1
35	Spathulenol	1579	1579	4.7 ± 1.0
36	Isospathulenol	1634	1634	0.4 ± 0.1
37	Hexadecanoic acid	1963	1963	1.2 ± 0.2
38	Suberosin	2148	2148	20.7 ± 3.1
	Monoterpene hydrocarbons			1.5
	Oxygenated monoterpenes			5.1
	Sesquiterpene hydrocarbons			29.8
	Oxygenated sesquiterpenes			5.1
	Non-terpene hydrocarbons			2.3
	Oxygenated non-terpenes			37.5
	Total identified			81.3

[a]Compounds listed in order of elution from HP-5MS column.
[b]Retention indices in literature.
[c]Retention indices to C_8–C_{24} *n*- alkanes on HP-5MS column.

and fruits (7.0%) essential oils, whereas its stereoisomer, *trans*-verbenol, was found as main compound of stems essential oil (22.1%). Thirty-eight compounds were also identified in the root essential oil, of which suberosin (7-methoxy-6-prenylcoumarin) (20.7%), together with β-barbatene (6.6%) and cuparene (6.1%) were found as main compounds (Table 2).

A review on the main compounds detected in the flowers, stems, leaves, fruits and roots of some *Ferulago* species during previous studies (Table 1)(8–20) and the results of present study on *F. trifida* reveals differences in relative amounts of the main compounds between various species of *Ferulago*. Among the common main compounds identified in the flowers, stems, leaves and fruits essential oils of *F. trifida*((E)-β-ocimene, α-pinene and bornyl acetate) (Table 3) α-pinene was the main compound in the most of previously analysed *Ferulago* species (Table 1), while (E)-β-ocimene and bornyl acetate were only found as main compounds in essential oils of *F. contracta* stems and *F. macrocarpa* fruits, respectively (Table 1).Ocimene has been also reported by Celik et al. (2013) as predominant compound (30.5%) in the essential oil of *F. sandrasica* leaves (19). 2,3,6-trimethylbenzaldehyde, a main compound reported from the fruits essential oils of *F. compestris*, *F. syriaca*, *F. asparagifolia*, *F. longistylis*, *F. asparagifolia* and *F. idaea* (Table 1) did not occur in the essential oil of *F. trifida* fruits. Suberosin, main compound of our root essential oil sample, was not detected in the essential oils of *F. compestris, F. isaurica* and *F. syriaca* roots (10,12). This volatile prenylated coumarin has been previously reported from the essential oils of *Cachrys cristata* fruits (19.7%), *Prango spabularia* fruits (1.8%) and *Angelica dahurica* roots (0.16%) (31–33). Differences in biochemical pathways caused by genetic diversities is the most possible factor involved in such differences observed between essential oil compositions of species belonging to the same genus. However, influence of extrinsic causes such as geographic factors (e.g. altitude, climate and soil properties), stresses caused by insects and microorganisms, plant harvesting time, drying and storage conditions, as well as essential oil extraction method cloud be considered as effective factors on mentioned differences are not negligible (33).

Antibacterial activity

The results of antibacterial activity assay of the essential oils have been shown in Table 4. As seen in Table 4, although the essential oil samples were inactive on *E. coli*, they exhibited a moderate (IZ: 10–15 mm) to very strong (IZ: >20 mm) antibacterial activity on the other seven bacterial strains (Table 4). Among the essential oils, antibacterial activity of the flowers essential oil on *S. epidermidis* (IZ: 19 mm, MIC: 125 μg ml^{-1}), stems essential oil on *S. epidermidis* (IZ: 34 mm, MIC: 125 μg ml^{-1}), *S. aureus* (IZ: 29 mm, MIC: 125 μg ml^{-1}) and *B. subtilis*(IZ: 22 mm, MIC: 250 μg ml^{-1}), leaves essential oil on *S. aureus* (IZ: 25 mm, MIC: 125 μg ml^{-1}) and fruits essential oil on *S. paratyphi* A (IZ: 25 mm, MIC: 125 μg ml^{-1}) were greater than positive control, gentamicin (IZ: 21 mm, MIC: 500 μg ml^{-1} for *S. aureus*, *S. paratyphi A* and *B. subtilis* and IZ: 18 mm, MIC: 500 μg ml^{-1} for *S. epidermidis*). Among the tree tested fungi, *A. brasiliensis* was only found susceptible to flowers, stems, leaves and roots essential oils (IZ: 11–12 mm). Essential oils obtained from the stem and flowers of *F. contracta* have been reported to possess very potent antibacterial activity (IZ: ≥20 mm, MIC:

Table 3. Chemical compositions of the flowers, stems, leaves and fruits essential oils of *F. trifida*.

				Real % Area			
No.	Compounds[a]	KI[b]	KI[c]	Flowers	Stems	Leaves	Fruits
1	α-Thujene	926	926	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
2	α-Pinene	934	934	16.3 ± 1.3	22.6 ± 1.4	19.6 ± 2.5	18.0 ± 2.1
3	Camphene	949	948	2.3 ± 0.2	2.4 ± 0.3	2.2 ± 0.1	2.6 ± 0.4
4	Verbenene	963	963	0.3 ± 0.2	0.8 ± 0.1	0.5 ± 0.1	0.2 ± 0.1
5	Sabinen	971	971	1.0 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	1.4 ± 0.2
6	β-Pinene	976	976	0.8 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	0.8 ± 0.3
7	β-Myrcene	991	990	2.8 ± 0.1	2.5 ± 0.4	3.2 ± 0.2	3.0 ± 0.4
8	α-Phellanderene	1005	1005	0.5 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.8 ± 0.2
9	α-Terpinene	1017	1016	0.1 ± 0.1	–	–	0.1 ± 0.1
10	*p*-Cymene	1020	1019	–	1.5 ± 0.2	2.5 ± 0.1	–
11	α-Limonene	1026	1026	4.3 ± 0.2	3.3 ± 0.1	3.3 ± 0.1	5.2 ± 0.4
12	(E)-β-Ocimene	1046	1046	37.3 ± 2.2	20.7 ± 2.4	25.7±3.3	30.5 ± 3.5
13	α-Ocimene	1051	1051	1.4 ± 0.3	0.7 ± 0.1	2.6 ± 0.1	1.4 ± 0.4
14	γ-Terpinene	1058	1058	7.5 ± 0.5	2.7 ± 0.2	4.2 ± 0.3	4.6 ± 1.1
15	α-Terpinolene	1089	1089	3.0 ± 0.2	–	0.7 ± 0.1	9.4 ± 2.6
16	Linalool B	1097	1097	0.2 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
17	Allo-Ocimene	1132	1131	1.2 ± 0.4	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2
18	Cis-Verbenol	1140	1139	8.6 ± 0.5	1.7 ± 0.3	10.4 ± 2.3	7.0 ± 0.4
19	Trans-Verbenol	1143	1143	–	22.1 ± 2.7	–	–
20	α-Phellandren-8-ol	1156	1157	–	2.5 ± 0.4	–	–
21	4-Terpineol	1165	1163	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2
22	p-Cymen-8-ol	1181	1181	–	0.2 ± 0.1	–	0.1 ± 0.1
23	a-Terpineol	1188	1188	–	0.1 ± 0.1	–	–
24	Verbenone	1208	1207	–	0.4 ± 0.1	0.2 ± 0.1	–
25	Thymol methyl ether	1235	1235	–	–	0.2 ± 0.1	–
26	Bornyl acetate	1289	1289	9.4 ± 1.0	8.5 ± 0.5	16.7 ± 2.0	11.0 ± 2.4
27	β-bourbenene	1378	1378	–	–	0.1 ± 0.1	–
28	Cis-Jasmone	1396	1396	–	–	0.2 ± 0.1	–
29	Trans-caryophellene	1458	1458	–	–	0.2 ± 0.1	–
30	GermacreneD	1487	1487	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2
31	β-bisabolene	1509	1509	–	0.1 ± 0.1	–	–
32	δ-Cadinene	1525	1526	0.2 ± 0.1	0.2 ± 0.1	–	0.1 ± 0.1
33	Caryophyllene oxide	1584	1585	–	–	0.1 ± 0.1	–
	Monoterpene hydrocarbons			79.1	60.6	68.3	79.2
	Oxygenated monoterpenes			9.1	27.8	11.0	7.9
	Sesquiterpene hydrocarbons			0.5	0.6	0.7	0.4
	Oxygenated sesquiterpenes			–	–	0.1	–
	Oxygenated non-terpenes			9.4	8.5	17.1	11.0
	Total identified			98.1	97.8	97.2	98.5

Note: A dash (–) indicate the absence of compound in the sample.
[a]Compounds listed in order of elution from HP-5MS column.
[b]Retention indices in literature.
[c]Retention indices to C_8–C_{24} *n*- alkanes on HP-5MS column.

31.25 μg ml^{-1}) on *Bacillus subtilus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* B (11).*Bacillus subtilis* was found as the most sensitive microorganism against the essential oil of *F. sandrasica* leaves with inhibition zones of 5 and 15 mm at 0.1 and 0.25 μg ml^{-1}, respectively (19). Some other *Ferulago* species such as *F. campestris*, *F. macedonia*, *F. longistylis* and *F. confuse* have been documented for antimicrobial activity of the essential oils obtained from their different parts (9,10,13,16 and 20). Considering to known antimicrobial activity of the (E)-β-ocimene, α-pinene and bornyl acetate(35,36), main compounds of the flowers, stems, leaves and fruits of *F. trifida* essential oils, these compounds may attributed to observed antimicrobial activity of related essential oil samples. Also, there are some reports on antimicrobial potentials of natural prenylated coumarins (37,38). Therefore, suberosin, the main compound of *F. trifida* roots essential oil, may be involved as an active principle in antimicrobial activity of the roots essential oil.

Free radical scavenging activity

Essential oils of the flowers, stems, leaves, fruits and roots of *F. trifida* exhibited weak free radical scavenging activity in DPPH method (IC_{50}: 95–120 μg ml^{-1}), in comparison with BHT, a commercial synthetic antioxidant (IC_{50}: 21.2 ± 2.6 μg ml^{-1}) (Table 5). This observed weak free radical scavenging activity may be related to the absence of free radical scavenger principles such as phenolic compounds in composition of tested essential oils (39).

Table 4. Antibacterial and antifungal activities of the flowers, stems, leaves and fruits essential oils of *F. trifida*.

	Essential oils										Antibiotics					
	Flowers		Stems		Leaves		Fruits		Roots		Rif[a]		Gen[b]		Nys[c]	
Microorganism	IZ[d]	MIC[e]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
S. paratyphi–A	16	500	16	500	17	500	25	125	19	250	–	–	21	500	NA[f]	NA
S. aureus	18	500	34	125	25	125	18	250	20	250	10	250	21	500	NA	NA
S. epidermidis	19	125	29	125	21	125	19	250	18	250	8	250	18	500	NA	NA
E. coli	–	–	–	–	–	–	–	–	–	–	11	500	20	500	NA	NA
K. pneumoniae	14	250	16	125	16	125	16	125	15	250	7	250	22	250	NA	NA
B. subtilis	18	125	22	250	18	125	10	250	11	250	13	15	21	500	NA	NA
P. vulgaris	12	250	13	500	15	500	14	250	14	250	10	125	23	500	NA	NA
S. dysenteriae	20	125	20	250	20	125	25	125	21	125	40	250	35	500	NA	NA
C. albicans	–	–	–	–	–	–	–	–	–	–	NA	NA	NA	NA	33	125
A. brasiliensis	11	500	12	125	12	125	–	–	11	500	NA	NA	NA	NA	23	500
A. niger	–	–	–	–	–	–	–	–	–	–	NA	NA	NA	NA	27	31

Note: A dash (–) indicates no antimicrobial activity.
[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^{-1}.
[f]Not applicable.

Table 5. The results of DPPH free radical scavenging, AChE inhibitory and brine shrimp lethality assays of the flowers, stems, leaves, fruits and roots essential oils of *F. trifida*.

Sample/Essential oil	DPPH free radical scavenging assay IC_{50} (µg ml^{-1})	AChE inhibitory assay Inhibition[a] (%)	Brine shrimp lethality test LD_{50} (µg ml^{-1})
Flowers	98.1 ± 5.2	72.1 ± 4.2	2.1 ± 0.3
Stems	119.7 ± 7.3	12.5 ± 2.3	3.4 ± 0.4
Leaves	113.1 ± 5.8	28.3 ± 3.1	1.1 ± 0.2
Fruits	102.3 ± 4.2	78.7 ± 5.2	1.6 ± 0.2
Roots	104.4 ± 3.7	74.3 ± 4.1	5.4 ± 0.3
BHT	21.2 ± 2.6	NA[b]	NA
Podophyllotoxin	NA	NA	2.8 ± 0.3

[a]The IC_{50} value of 0.015 µg ml^{-1} was determined for donepezil in AChE inhibitory assay, as positive control.
[b]Not applicable.

Table 6. The results of MTT assay of the flowers, stems, leaves, fruits and roots essential oils of *F. trifida* on different cell lines.

	Cell lines (IC_{50}/µg ml^{-1})		
Sample/Essential oil	MCF7[a]	A-549[b]	HT-29[c]
Flowers	27.0	26.5	40.55
Stems	24.0	35.6	49.4
Leaves	24.7	15.4	35.5
Fruits	27.0	25.8	54.3
Roots	25.1	40.1	38.8
Tomoxifen	3.6	10.7	2.50

[a]human breast adenocarcinoma.
[b]non-small cell line carcinoma.
[c]human colon adenocarcinoma.

AChE inhibitory activity

The results of AChE inhibitory activity of the essential oils have been shown in Table 5. Flowers, fruits and roots essential oils, demonstrated considerable AChE inhibitory activity (72.1, 78.7 and 74.3% inhibition of Ach Eenzyme, respectively). In agreement of our results, Hritcu et al. (2015) reported that the essential oil obtained from flowering aerial parts of *F. angulata* possessed antiamnesic effect in scopolamine-induced memory impairments in rats. They showed that the essential oil of *F. angulate* ameliorate memory impairments through decrease in hippocampal AChE activity and neuroprotection against scopolamine-induced oxidative stress in the rat hippocampus (21).

Brine shrimp lethality activity

General toxicity potentials of the essential oil samples were evaluated against *A. salina* larva in brine shrimp

lethality test. As shown in Table 5, the essential oils exhibited considerable toxicity with LD_{50} ranging from 1.1 to 5.4 μg ml^{-1}. Among the tested essential oils, toxicity of the flowers (2.1 ± 0.3 μg ml^{-1}), leaves (1.1 ± 0.2 μg ml^{-1}) and fruits (1.6 ± 0.2 μg ml^{-1}) essential oils were found higher than positive control, podophyllotoxin (2.8 ± 0.3 μg ml^{-1}). Literature review revealed that there is no any report on brine shrimp lethality activity of *Ferulago* essential oils. However, general toxicity assay of some Turkish *Ferulago* species by Gurkan et al. (1995) showed that chloroform and ethanol extracts of the whole plant of *F. aucheri* had the highest toxicity effects with LD_{50} value of 30.77 μg ml^{-1}in BSLT (40).

In vitro *cytotoxic activity*

The results of MTT assay of essential oils on three cancerous cell lines, MCF-7, A-549 and HT-29 have been summarized in Table 6. As seen in Table 6, among the samples, leaves essential oil exhibited the highest cytotoxic effect on A-549 with IC_{50} value of 15.39 μg ml^{-1}. Despite observed potent toxicity effects in BSLT (Table 5), essential oils showed approximately moderate cytotoxic activity on the tested cell lines (IC_{50}: 15–55 μg ml^{-1}) (Table 6). Although BSLT is commonly used as a proper primary assay for screening of cytotoxic potentials of plant extracts, some studies found no correlation between *A. salina* lethality and cell lines growth inhibition. They supposed that toxicity effect could be exerted by different mechanisms in the complex cellular organism and an eukaryotic cell (41–43).

In conclusion, the results of this study suggest the essential oils of *F. trifida* as sources of bioactive compounds with considerable antibacterial and AChE inhibitory effects. Potent antibacterial activity of *F. trifida* essential oils, make it an appropriate option to use as a natural flavour and preservative in food industries. However, further toxicological studies are necessary to evaluate its safety perspectives.

Acknowledgement

The authors are grateful to Dr M. Agha ahmadi (Faculty of Science, Department of Biology, University of Isfahan, Isfahan, Iran) for his kind help in collection of the plant and Professor V. Mozaffarian (Research Institute of Forest and Rangelands, Tehran, Iran) for identification of plant.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Tehran University of Medical Sciences and Health Services [grant number 29402].

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Chemical composition and biological activities of the essential oils from different parts of Ferulago trifida Boiss

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Data from the 2016 edition of Journal Citation Reports

Publisher
TAYLOR & FRANCIS INC, 530 WALNUT STREET, STE 850, PHILADELPHIA, PA 19106 USA

ISSN: 1041-2905
eISSN: 2163-8152

Research Domain
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Journal

Journal of Essential Oil Research ›

Volume 29, 2017 - Issue 5

Original Articles

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Pages 407-419 | Received 07 Mar 2016, Accepted 06 Mar 2017, Published online: 17 Apr 2017

Download citation https://doi.org/10.1080/10412905.2017.1313178

Abstract

As a result of GC and GC-MS analysis of the essential oils from different parts of *Ferulago trifida* Boiss., a total of thirty-three compounds were identified in the flowers

(Fl), stems (S), leaves (L) and fruits (Fr) essential oils, of which (E)-β-ocimene (Fl: 37.3, S: 20.7, L: 25.7, Fr: 30.5%), α-pinene (Fl: 16.3, S: 22.6, L: 19.6, Fr: 18.0%) and bornyl acetate

Journal of Essential Oil Research

2016 Impact Factor
0.972