

Chemical composition and bioactivities of the essential oil from different organs of *Ferula communis* L. growing in Tunisia

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Abstract The essential oils obtained by the hydrodistillation from the fresh flowers, leaves, stems, and roots of *Ferula communis* L., growing in Tunisia were analyzed by GC and GC/MS. Thirty-two components were identified in the oil of flowers with camphor (18.3 %), α -pinene (15.3 %), and β -eudesmol (9.3 %) as the main constituents. Twenty-nine compounds were identified in the oil of stems with β -eudesmol (28.1 %), δ -eudesmol (11.1 %), and α -eudesmol (9.6 %) as the main compounds. Twenty compounds were characterized in the oil of roots with dillapiol (7.9 %), guaiol (7.3 %), and spathulenol (6.8 %). In the oil of leaves, α -eudesmol (25.2 %), β -eudesmol (20.7 %), δ -eudesmol (10.1 %), and caryophyllene oxide (7.2 %) were found as the main constituents. This study was undertaken to evaluate the antioxidant activity using DPPH (2,2'-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid), reducing power, and catalase activity. We tested also the antibacterial, cytotoxic, and cholinesterase inhibition properties of the essential oil of different organs of *F. communis*. The essential oil of the stems showed the highest antioxidant

activity ($IC_{50} = 0.03 \pm 0.001$ mg mL⁻¹), in DPPH assay and the important result of catalase (303.03 μ mol H₂O₂ degraded/min/protein) of *F. communis*. The antibacterial activity of the oil was determined by micro-well dilution assay. The best results (MIC = 0.156 ± 0.02 mg mL⁻¹) were exhibited by the essential oil of the leaves of *F. Communis* against *Pseudomonas aeruginosa*. Besides, the strongest cytotoxic activity against Hela cells was shown with essential oils' leaves with an inhibition percentage of 79.05 % at the concentration of 500 μ g mL⁻¹. However, the best inhibition percentage of A 549 cells was detected for essential oils' leaves with an inhibition percentage of 54.56 % at 250 μ g mL⁻¹. Our finding showed that the essential oil of the flowers was the most active, with 64.623 % of inhibition against butyrylcholinesterase at 10 mg mL⁻¹ from the incubation time of 30 min.

Keywords *Ferula communis* L. · Antioxidant · Antibacterial · Cytotoxic and anticholinesterase activities · Essential oil

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Introduction

The family of Apiaceae contains an extremely wide variety of aromatic plants. Among this rich array of plants yielding essential oils, the genus *Ferula communis* L. distributed worldwide is very important, including 172 species (Stravroula *et al.*, 2013) that are spread around naturally on the pasture of the Eastern Mediterranean, and goat flocks grazed it with other herbaceous vegetation. The Tunisian flora comprises four species: *F. communis*, *Ferula tingitana*, *Ferula tunetana* (Jabrane *et al.*, 2010a), and *Ferula lutea* (Znati *et al.*, 2012). *F. communis* has got green vegetation during April and May and then gets dry. *Ferula*

plants include phytoestrogenic substances that affect reproductive hormones such as progesterone, testosterone, and estrogens (Aragno *et al.*, 1988). The active substances of phytoestrogen are isoflavones and ferutin (Valle *et al.*, 1987; Appendino *et al.*, 2001). *Ferula* varieties also include some terpene and terpenoid compounds (<http://pubs.acs.org/action/doSearch?action=search&author=Shikishima%2C+Y&qsearchArea=author> Nagatsu *et al.*, 2002).

In eastern Mediterranean mountainous regions of Turkey, some people eat ground *F. communis* root by mixing it with honey. Goat keepers and other citizens of the region claim that this plant has an aphrodisiac role for both animals and humans (Keskin *et al.*, 2004).

This species possesses several traditional medicinal properties. It is used for skin diseases, rheumatism, cracks in the feet, helminthic disease, pains in the joints, hysteria, and dysentery. It is antispasmodic, vermifugal, and aphrodisiac and can be used against moths or ringworm and as a depilatory. In Morocco *F. communis* is frequently used to treat various diseases, and orally it is prescribed by practitioners as an anti-helminthic, diuretic, analgesic, for pains in the joints, female sterility, rheumatism, and an emetic. The roots are also used for hair care (Bellakhdar, 1978).

Chemical composition studies of *F. communis* showed that it contains 4-hydroxycoumarin derivatives, some of which were demonstrated to have hypothermic action. 4-Hydroxycoumarin anticoagulants are known (Nicholas and Rettie, 2008), on the other hand, to be used for the treatment and management of thromboembolic disease in humans. In this study we try to evaluate the cholinesterase inhibition properties of the essential oils of *F. communis*. Alzheimer's disease is the cause of a major cholinergic deficit, particularly neurotransmitter involved in attention and memory.

One way to increase the activity of the cholinergic system is to avoid the breakdown of acetylcholine by blocking cholinesterase. There are two types of cholinesterase enzyme: acetylcholinesterase (AChE), also known as acetylcholine. The acetylhydrolase is found primarily in the blood and neural synapses and pseudocholinesterase. The second one butyrylcholinesterase (BChE or BuChE), also known as plasma cholinesterase, is found primarily in the liver, and it serves as a model of the predominant treatment used in Alzheimer's disease. In this frame, plants can be a source of inhibitors of acetylcholinesterase. In fact, from 1981 to 2006, 25 % of new anti-Alzheimer's molecules were shown to be of natural origin (Newman and Cragg, 2007).

To enhance further our work, we studied the catalase antioxidant activity. Catalase is a metalloenzyme which dismutates hydrogen peroxide to water and oxygen. It is widely distributed in animals, plants, and all aerobic

microorganisms. Catalase is an enzyme present in most of the aerobic cells, and it protects them from oxidative stress (inflammatory, tumor, diabetes, cardiovascular diseases, anemia and Wilson's disease) (Al-Abrash *et al.*, 2000).

Given a great interest in novel natural compounds able to fight against Alzheimer's, cancer and many other diseases, we undertook the present study as a primary biological investigation of the anticholinesterase activity, cytotoxic, antioxidant, and antibacterial effects of different organs of *F. communis* L. essential oils.

Materials and methods

Plant material

Ferula communis L. was collected in April 2010 in Grombalia (Tunisia). The identification was performed at the laboratory of Plant Biology and Botanic, the High Institute of Biotechnology of Monastir, the University of Monastir, Tunisia. A voucher specimen (C.M-10) has been deposited in our laboratory.

Extraction of the essential oils

Fresh flowers, leaves, stems, and roots were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. The obtained essential oils were collected by decantation, dried over sodium sulfate, filtered, and stored at 4 °C until analyzed.

Analytical GC

Gas chromatographic analysis was carried out on a HP-5890 gas chromatograph fitted with a HP-Wax and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness). The GC oven temperature was programmed at 60 °C (held for 10 min) and heated to 220 °C at 5 °C/min. The injector and detector temperatures were maintained at 250 °C. Helium was used as carrier gas at a flow rate of 2 mL/min. Volume injected was 0.1 μL of 1 % hexane solution. The identification of the components was performed by comparing their retention times with those of pure authentic samples and by mean of their linear retention indices (L.R.I.) relative to the series of *n*-hydrocarbons.

Analytical GC–MS

GC/EIMS analysis were performed with a Varian CP-3800 gas chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion-trap mass detector. Analytical

conditions are: injector and transfer line temperatures 220 and 240 °C, respectively; the oven's temperature programmed from 60 to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10 % hexane solution); split ratio 1:30. The identification of the constituents was based on the comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Stenhagen *et al.*, 1974; Massada, 1976; Jennings and Shibamoto, 1980; Davies, 1990; Swigar and Silverstein, 1981; Adams, 2007). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing.

Antioxidant activity

DPPH radical-scavenging assay

The antioxidant activity of the essential oil of different organs of *F. communis* L. was determined by the slightly modified method of Hatano *et al.* (1988).

In total, 0.5 mL of each sample concentration was mixed with the same volume of DPPH ethanol solution. After 30-min incubation in the darkness at 25 °C, the absorbance of the sample at 520 nm was read. A mixture of 0.5 mL of DPPH solution and 0.5 mL of ethanol was used as a blank. The decrease in absorption induced by the samples was compared to that of the positive control, BHT (butylated hydroxytoluene). The calculated IC₅₀ values denoted the concentration required to scavenge 50 % of DPPH radicals. The results were expressed in an inhibited percentage versus samples' concentrations (mg mL⁻¹) at 30 min. All the measurements were performed in triplicate.

ABTS radical-scavenging assay

The radical-scavenging capacity of antioxidant for the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical was determined as described by Re *et al.* (1999). ABTS was generated by mixing 7 mM of ABTS at pH 7.4 (5 mM NaH₂PO₄, 5 mM Na₂HPO₄ and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by a storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using a spectrophotometer (Helios, Unicam, Cambridge, UK). For each sample, the diluted methanol solution of essential oil (100 µL) was allowed to react with fresh ABTS solution (900 µL), and then the absorbance was measured 6 min after initial mixing.

BHT (butylated hydroxytoluene) was used as a positive standard. The capacity of free radical scavenging was expressed as IC₅₀ (mg/L) value, which represents the concentration required to scavenge 50 % of ABTS radicals. The capacity of free radical scavenging IC₅₀ was determined using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

Reducing power assay

The ferric-reducing power of the extracts and references was tested using the assay of Oyaizu (1986). A total of 1 mL of different concentrations of the extracts as well as chlorogenic acid as reference for comparative purposes was added to 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide. Later, the mixture was incubated at 50 °C for 20 min and then 10 % trichloroacetic acid was added. After the mixture was shaken vigorously, this solution was mixed with distilled water and FeCl₃ (0.1 %, w/v). After 30 min of incubation, the absorbance was read at 700 nm. The analyses were achieved in duplicate. The increased absorbance of the reaction meant increased reducing power.

Catalase activity

The catalase activity was measured according to Aebi's (1984) method. Hydrogen peroxide (H₂O₂) disappearance was monitored kinetically at 240 nm for 1 min at 25 °C. The enzyme activity was calculated using an extinction coefficient of 0.043 mM⁻¹ cm⁻¹. One unit of activity is equal to 1 µmol of H₂O₂ destroyed/min/mg protein.

Antibacterial activity

Microorganisms

The antibacterial activity was tested against five microorganisms, including reference strains consisting of gram-negative rods: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), gram-positive cocci: *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212), and clinical strains: *Acinetobacter* sp. The bacterial strains were cultured over night at 37 °C in Muller Hinton agar.

Micro-well dilution assay

This methodology is applied in order to establish minimum inhibitory concentrations (MIC) of different essential oils, against bacteria.

The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. MIC values were determined by a microtiter plate dilution method Jabrane *et al.* (2010b) dissolving the sample in 10 % DMSO solution. Sterile 10 % DMSO solution (100 μL) was pipette into all wells of the micro-titer plate before transferring 100 μL of stock solution to the microplate. Serial dilutions were made to obtain a concentration ranging from 10 to 0.0775 mg mL^{-1} . Finally, 50 μL of 10^6 colony-forming units (cfu/mL) (according to Mc Farland turbidity standards) of standards microorganism suspensions were inoculated on to microplates and incubated at 37 °C for 24 h. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the sample at which the absence of growth was recorded. All the samples were screened three times against each microorganism. The imipenem was employed as a positive control against gram-positive and gram-negative bacteria. The final concentration of DMSO in the well had no effect on bacterial growth.

Anticholinesterase activity

The anticholinesterase activity was studied by the colorimetric method of Ellman *et al.* (1961) with a slightly modification. The butyrylcholinesterase (BchE) hydrolyzed the butyrylthiocholine (colorless) to butyrate and thiocholine.

Thiocholine reacts with DTNB to form 5-mercapto-2-nitrobenzoic acid (5-MNBA) which is a yellow product. The rate of 5-MNBA formation, measured spectrophotometrically at 405 nm, is proportional to the enzymatic activity of BchE in the sample.

Human plasma (pool plasma from samples designated for biochemical analysis) was used as a source of BchE. In total, 100 μL of extract were added to 100 μL of plasma, and the mixture was incubated at 37 °C for a determined time. After incubation, the enzyme's activity was measured by Konelab 30[®] apparatus (1 cm cuvette). The control (plasma and distilled water) was treated in the same conditions. All assays were carried out in duplicate. The anticholinesterase activity was calculated by the following formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Activity of control} - \text{Activity of sample}}{\text{Activity of control}} \right] \times 100,$$

IC_{50} as the sample concentration providing 50 % inhibition (IC_{50}) To determine the effect of temperature and incubation times, the assays were carried by the same procedure for the same extract, at 25 °C (fixed incubation times) and for different times at 37 °C.

Cytotoxic activity

Cell cultures

The human A549 lung epithelial carcinoma and Hela cervix cells lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's modified Eagle medium (DMEM, Gibco) containing 10 % (v/v) fetal calf serum, 2 mM glutamine, and antibiotics (200 U L^{-1} of penicillin and 50 mg L^{-1} of streptomycin). The cells were maintained at 37 °C in a humidified 5 % CO_2 atmosphere.

Cell viability assay

The cytotoxicity was measured by using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test with slight modifications (Yan *et al.*, 2009). The cells were seeded at 5×10^3 cells/well in 100 μL of growth medium and incubated at 37 °C for 24 h to adhere. The cells were treated by essential oils and incubated for 48 h; then 10 μL of MTT (5 mg mL^{-1}) was added to each well and the incubation lasted 2 h. After this, 100 μL of DMSO were added to each well. The absorbance (A) was measured at 540 nm by a Multiskan Ascent (Ascent Software version 2.6) microplate reader. This assay was conducted in triplicate and the percentages of cell growth were calculated as follow:

$$\text{Cell growth}(\%) = \left[\frac{A(\text{sample})}{A(\text{control})} \right] \times 100.$$

Cytotoxicity was expressed as the concentration of extract inhibiting cell's growth by 50 %.

Results and discussion

Chemical composition

The hydrodistillation of the dried aerial's parts (flowers, leaves, and stems), roots of *F. communis*, gave yellow essential oils in yields ranging from 0.024 % (roots), 0.11 % (leaves), 0.15 % (stems) to 0.18 % (flowers).

The chemical compositions of the oils were analyzed by GC and GC-MS. More than 20 constituents were identified according to their retention indices (L.R.I). The identified compounds are presented in Table 1, and some significant differences were noted in the quantitative and qualitative composition of the oils obtained from stems and leaves. The main constituents of both oils were sesquiterpenoids (56 % and 48.8 %, respectively) with α -, β -, γ -eudesmol.

The oil obtained from flowers of *F. communis* present 32 compounds, which represented 97.3 % of the total amount of the oil, containing mainly camphor (18.3 %), α -pinene

Table 1 Chemical composition of the essential oils of *F. communis* L. (roots, leaves, stems and flowers)

Compounds	L.R.I ^a	L.R.I ^b	<i>F</i> ₁	<i>F</i> ₂	<i>F</i> ₃	<i>F</i> ₄
α -Pinene	941	1028	15.3	–	–	–
Camphene	955	1067	0.7	–	–	–
β -Pinene	982	1108	4.4	–	–	–
Myrcene	993	1166	5.0	–	–	–
δ -3-Carene	1012	1144	0.9	–	–	–
<i>p</i> -Cymene	1028	1242	0.6	–	–	–
Limonene	1032	1198	2.1	–	–	–
1,8-Cineole	1034	1204	1.0	–	–	–
Linalool	1101	1560	1.2	1.3	–	–
<i>Trans</i> -pinocarveol	1140	1635	0.5	–	–	–
Camphor	1145	1522	18.3	–	–	–
Borneol	1168	1796	0.6	–	–	–
<i>p</i> -Cymen-8-ol	1185	1833	1.0	–	–	–
Verbenone	1206	1716	0.6	–	–	–
<i>p</i> -Vinyl guaiacol	1315	2184	–	–	1.3	–
Cyclosativene	1370	1480	–	1.7	–	0.6
β -Caryophyllene	1420	1598	0.5	–	–	0.6
β -Gurjunene	1433	1601	–	–	3.4	–
(<i>E</i>)-geranylacetone	1455	1842	–	–	2.2	–
(<i>E</i>)- β -farnesene	1460	1661	–	–	4.7	–
γ -Muurolene	1478	1681	–	1.1	1.7	0.6
α -Amorphene	1483	1668	–	1.5	–	1.2
<i>ar</i> -Curcumene	1484	1786	1.7	–	–	0.7
β -Selinene	1487	1715	–	2.0	2.0	1.0
Valencene	1493	1717	–	0.8	1.5	0.7
β -Dihydroagarofuran	1499	1708	1.1	1.0	–	1.0
<i>Trans</i> - β -guaiene	1501	1829	–	–	1.2	–
<i>Cis</i> - γ -cadinene	1511	1749	–	–	1.3	–
<i>Trans</i> - γ -cadinene	1514	1750	–	1.1	1.5	0.5
Myristicin	1520	2290	–	–	6.0	–
<i>Trans</i> -cadinane-1(2)-4-diene	1535	–	–	–	1.2	–
α -Agarofuran	1548	1875	–	–	2.1	–
Elemol	1552	2085	2.8	3.0	–	2.6
Germacrene B	1558	1870	–	2.9	–	–
(<i>E</i>)-nerolidol	1565	2006	–	–	3.5	–
Dimethyl-ionone	1566	–	2.7	–	–	1.5
Spathulenol	1577	2137	–	–	6.8	–
Caryophyllene oxide	1582	1966	8.0	3.4	–	7.2
Globulol	1585	2056	–	–	2.6	–
Carotol	1596	2027	1.6	–	–	–
Guaiol	1598	2101	–	2.1	7.3	3.0
Geranyl 2-methylbutyrate	1600	1901	–	1.2	–	–
5-Epi-7- <i>epi</i> - α -cubenol	1607	–	–	–	1.0	–
Humulene epoxide II	1607	2069	0.9	–	–	1.1
1,10-Di- <i>epi</i> -cubenol	1616	2045	–	–	1.3	–
10- <i>Epi</i> - γ -eudesmol	1620	2111	1.1	1.2	1.4	1.5
Dillapiole	1622	2348	–	1.6	7.9	0.8
Eremoligenol	1630	2205	1.2	3.6	–	3.4

Table 1 continued

Compounds	L.R.I. ^a	L.R.I. ^b	F ₁	F ₂	F ₃	F ₄
γ-Eudesmol	1632	2188	4.6	11.1	–	10.1
Hinesol	1640	2189	–	–	–	2.4
T-Cadinol	1642	2139	0.6	1.7	5.9	1.4
T-Muurolol	1644	2150	0.9	1.4	3.0	–
β-Eudesmol	1650	2247	9.3	28.1	–	20.7
α-Eudesmol	1653	2233	3.6	9.6	–	25.2
α-Cadinol	1655	2186	–	–	4.4	–
5-Hydroxy-isobornyl isobutyrate	1656	–	1.0	2.1	–	1.0
Cis-calamenen-10-ol	1660	2354	–	0.8	–	1.2
Bulnesol	1667	2211	–	–	1.2	–
Cadalene	1675	2248	–	0.8	1.7	–
10-Nor-calamenen-10-one	1703	2422	–	0.8	–	–
(E)-nerolidol acetate	1716	–	–	–	3.4	–
Oplopanone	1737	2548	2.7	1.9	–	1.2
(E,Z)-farnesol	1744	2327	–	–	2.0	–
(Z)-lanceol	1761	2513	–	–	3.1	0.6
Benzyl benzoate	1762	2655	0.8	–	–	–
γ-Eudesmol acetate	1780	2180	–	–	1.3	–
α-Eudesmol acetate	1791	–	–	–	1.2	–
8-α-acetoxyelemol	1792	2480	–	–	1.3	–
Cryptomeridiol	1810	2603	–	1.0	–	–
Carissone	1926	2620	–	–	–	1.0
Methyl hexadecanoate	1928	2210	–	1.8	–	1.9
Ethyl palmitate	1994	–	–	0.7	–	–
Total identified			97.3	91.3	90.4	94.7

F₁, Flowers; F₂, Stems; F₃, Roots; F₄, Leaves

^a Order of elution and percentages of individual components are given on a HP-5 capillary column

^b l.r.i. on HP-Wax column

(15.3 %), β-eudesmol (9.3 %), caryophyllene oxide (8.0 %), and myrcene (5.0 %).

The dillapiole (7.9 %) was found as the major component of root's essential oil, followed by guaiol (7.3 %), spathulenol (6.8 %), myristicin (6.0 %), and T-cadinol (5.9 %).

The present essential oil is also characterized by the presence of sesquiterpenoids compounds only; the essential oil of the flowers presents camphor (18.3 %) and α-pinene (15.3 %) as major compounds. The last constituents seem to be the main component of the essential oils of *F. communis* ssp *glauca* (leaves and flowers, respectively) (Maggi *et al.*, 2009), *F. lycia* (Kose *et al.*, 2010), *F. badrakema* (Asili *et al.*, 2009), *F. szovitsiana* (Dehghan *et al.*, 2007), *F. ovina* (Ghannadi *et al.*, 2002), *F. gummosa* (gum and latex) (Ghannadi and Amree, 2002), *F. gummosa* (fruits) (Sayyah *et al.*, 2001), *F. flabelliloba* (Rustaiyan *et al.*, 2001a), *F. stenocarpa* (Rustaiyan *et al.*, 2001b), *F. jae-sekheana* (collected in May), *F. jae-sekheana* (collected in July) (Kapahi *et al.*, 1985), and *F. penninervis* (Goryaev

et al., 1968) (11.7, 24.2, 59.9, 10.9, 8.0, 5.7, 18.3, 10.0, 48.8, 9.5, 30.0, 4.7 %, respectively).

Literarily, the composition of *F. communis* volatiles from the Mediterranean area has been reported: The leaves' oil from Corsica was characterized by the presence of myrcene (53.5 %) and aristolene (8.5 %) (Ferrari *et al.*, 2005); in the inflorescences oil from Sardinia, the main metabolites were the sesquiterpenes α- and β-gurjunene (40.7 and 7.1 %, respectively) (Marongiu *et al.*, 2005); the oils from the aerial parts of the Sardinian populations had as major volatiles in the poisonous chemotype the sesquiterpenes aristolene (47.1 %) and (E,E)-farnesol (21.2 %), while in the non-poisonous chemotype, the main component was the sesquiterpene allohedycaryol (53.7 %) (Rubiolo *et al.*, 2006). The essential oil's composition of *F. glauca* (formerly considered as subspecies of *F. communis*) from central Italy appear to be different having as major volatiles in leaves (E)-caryophyllene and caryophyllene oxide, in flowers α-pinene, myrcene, and germacrene D and in fruits α- and β-pinene (Maggi *et al.*, 2009).

The essential oils of Greek *F. communis* subsp. *Communis* from the leaves collected from three different regions in Chania (Crete) at the flowering stage (May 2007) (samples le1, infl1: 3 km SW Chania) and at the fruiting stage (April 2008) (samples le2, infr2: along the road between airport and Ag. Triada, samples le3, infr3: 5 km SW Chania) were characterized by the abundance of sesquiterpenes (85.7–88.7 %). Specifically, in sample le1 the main constituents were α - and β -eudesmol (12.6, 9.7 %), in sample le2 δ -cadinene (10.8 %) and germacrene B (10.1 %) and in the sample le 3 α -eudesmol (16.1 %) and δ - and γ -cadinene (13.6, 12.5 %). The inflorescences' oil (sample infl1) was also abundant in sesquiterpenes with γ - and *ar*-curcumene (14.0, 8.5 %) being the main ones, while γ -terpinene (10.8 %) was the major component in monoterpenes's fraction. on the contrary the infructescences oils (samples infr2, infr3) possessed high levels of monoterpenes, with α -pinene (35.2 and 13.6 %, respectively) and β -pinene (40.6, 10.2 %) being the major compounds (Manolakou *et al.*, 2013).

Antioxidant activity

Free radical scavenging activity (DPPH assay)

The antioxidant measurement by DPPH method showed that the essential oil of roots exhibited a moderate activity ($IC_{50} = 0.068 \pm 0.003 \text{ mg mL}^{-1}$) than flowers' essential oil ($IC_{50} = 0.060 \pm 0.001 \text{ mg mL}^{-1}$). Stems' essential oil is the most potent radical scavenger with $IC_{50} = 0.03 \pm 0.001 \text{ mg mL}^{-1}$, than leaves ($IC_{50} = 0.030 \pm 0.002 \text{ mg mL}^{-1}$). The essential oil of *F. communis* has an antioxidant activity that is related to its chemical composition. The germacrene B which is present only in relative amounts in stems' oil can be partly responsible for such activity, but it is difficult to attribute this activity to a single compound as a synergy between the different compounds that can occur.

ABTS radical-scavenging assay

According to the ABTS assay results, the essential oils of the four parts of *F. communis* showed weak antioxidant activity. The most active essential oil plant's part was the stem, with an IC_{50} value of $0.229 \pm 0.01 \text{ mg mL}^{-1}$, followed by the leaves ($IC_{50} = 0.26 \pm 0.01 \text{ mg mL}^{-1}$), then the flowers with $IC_{50} = 0.51 \pm 0.03 \text{ mg mL}^{-1}$ and the roots ($IC_{50} = 0.569 \pm 0.02 \text{ mg mL}^{-1}$).

Reducing power assay

Another method to evaluate the antioxidant ability is based on the reduction of Fe^{3+} to Fe^{2+} in which the yellow color

of the test solution changes to various shades of green and blue, depending on the reducing power of each sample. The presence of the reducing agents causes the conversion of Fe^{3+} /ferricyanide complex to the ferrous form that may be followed at 700 nm due to the formation of Perl's Prussian blue $Fe_4[Fe(CN)_6]_3$. Increasing the absorbance at 700 nm indicates an increase in the reductive ability (Joshi *et al.*, 2010). The values of the reducing power at different organs of essential oils (flowers, leaves, stems and roots) of *F. communis* are shown in Table 2. The antioxidant activity of *F. communis* stem oils showed moderate ability for scavenging DPPH free radicals, when compared to this reference of (BHT). As well as, the same oils had the highest antioxidant capacity ($IC_{50} = 0.097 \pm 0.01 \text{ mg mL}^{-1}$) relative to leaves ($IC_{50} = 0.234 \pm 0.01 \text{ mg mL}^{-1}$), flowers ($IC_{50} = 0.253 \pm 0.002 \text{ mg mL}^{-1}$), and roots ($IC_{50} = 0.466 \pm 0.02 \text{ mg mL}^{-1}$, which had the weakest activity against the three antioxidant assays), when measured through the reducing power's assay.

Catalase activity

In the present study we demonstrated that the activity of the enzyme catalase from stems' essential oils (303.03 units) was significantly higher compared with that in leaves (151.51 units). The decrease in catalase activity may cause the accumulation of the O_2^- , H_2O_2 or accumulation of hydrogen peroxide correlates with cancer metastasis (Tsai *et al.*, 2014). Loss of catalase activity results in oxygen intolerance and triggers a number of deleterious reactions such as protein and DNA's oxidation, and cell's death (Halliwell and Gutteridge, 1999). The catalase plays a protective role against cardiovascular diseases.

These results confirm the correlation between our results in antioxidant activities: Stems show an interesting DPPH, ABTS, reducing power, and catalase activities, followed by the leaves of *F. communis*'s essential oils.

Antibacterial activity

As far as the in vitro antibacterial activity results are concerned, in general the essential oils possessed extremely moderate activity potential (Table 3).

In the presence of leaves *F. communis* essential oils, the strongest activity was observed against *P. aeruginosa* with an MIC at 0.156 mg mL^{-1} , followed by *Acintobacter*, *S. aureus*, and *E. coli*, with MIC at 0.31; 2.5; and 5 mg mL^{-1} , respectively. The weakest activity was observed against *E. faecalis* (MIC = 10 mg mL^{-1}). Stems' essential oils were active against *P. aeruginosa* and *Acintobacter* with MIC = 0.312 mg mL^{-1} and weakly active against *S. aureus* and *E. faecalis* (MIC = 10 mg mL^{-1}) and had no effect on the growth of *E. coli*. The most prominent

Table 2 Antioxidant activity of flowers, leaves, stems, and roots essential oils of *Ferula communis* L.

Sample	DPPH radical IC ₅₀ (mg mL ⁻¹) ^a	ABTS ⁺ radical IC ₅₀ (mg mL ⁻¹) ^a	Reducing power IC ₅₀ (mg mL ⁻¹) ^a	Catalase (μmol H ₂ O ₂ degraded/min/ protein)
Flowers	0.06 ± 0.001	0.51 ± 0.03	0.253 ± 0.002	3.0 ± 0.001
Leaves	0.035 ± 0.002	0.26 ± 0.01	0.234 ± 0.01	151.51 ± 0.01
Stems	0.03 ± 0.001	0.229 ± 0.01	0.097 ± 0.01	303.03 ± 0.002
Roots	0.068 ± 0.003	0.569 ± 0.02	0.466 ± 0.02	1.51 ± 0.001
BHT ^b	0.018 ± 0.01	0.050 ± 0.01	0.02 ± 0.01	–
Vitamin C	–	–	–	757.575 ± 0.002

Values were expressed as mean ± SE

^a IC₅₀ (mg mL⁻¹) the concentration at which 50 % is inhibited

^b BHT butylated hydroxytoluene

Table 3 Minimum inhibitory concentration (MIC) of flowers, leaves, stems, and roots essential oils of *F. communis* L.

Bacteria	MIC ^a (mg mL ⁻¹)					
	Flowers	Leaves	Stems	Roots	Imip.	Vancomycine
<i>Escherichia coli</i> ATCC 25922 ^c	5.0 ± 0.01 ^b	5.0 ± 0.01 ^b	>10	1.25 ± 0.02 ^b	–	0.00195
<i>Staphylococcus aureus</i> ATCC ^c 25923	5.0 ± 0.01 ^b	2.5 ± 0.01 ^b	10 ± 0.2 ^b	2.5 ± 0.04 ^b	–	0.0039
<i>Enterococcus faecalis</i> ATCC 29212	>10	10 ± 0.01 ^b	10 ± 0.1 ^b	5.0 ± 0.05 ^b	–	0.0625
<i>Pseudomonas aeruginosa</i> ATCC 27950	1.25 ± 0.2 ^b	0.156 ± 0.02 ^b	0.312 ± 0.01 ^b	>10	0.00195	–
<i>Acintobacter</i>	5.0 ± 0.02 ^b	0.31 ± 0.003 ^b	0.312 ± 0.01 ^b	5.0 ± 0.1 ^b	–	–

Values are expressed as mean ± SD

(–) no activity

ATCC American Type Culture Collection

Imip imipenem as antibiotic

^a MIC minimum inhibitory concentration (mg mL⁻¹)

^b Values with different letters differ significantly at $p < 0.05$

inhibitory action of roots oil was observed against *E. coli* with MIC = 1.25 mg mL⁻¹. However, the *S. aureus* showed weak activity with MIC values of 2.5 mg mL⁻¹. The results show also that flowers' essential oils were active only against *P. aeruginosa* (MIC = 1.25 mg mL⁻¹). The essential oils evaluated in this study presented a great variety of sesquiterpenes that could be considered as answerable for the antimicrobial activity (Maggi *et al.*, 2009; Del-Vechio-Vieira *et al.*, 2009; Chalchat and Garry, 1997). The antibacterial activity of different organs of *F. communis* essential oils could be associated with the presence of oxygenated sesquiterpenes components, and α - and β -eudesmol are well-known substances with pronounced antimicrobial properties (Yu *et al.*, 2008).

Anticholinesterase activity

Plasma cholinesterase was butyrylcholinesterase (BchE) or pseudo-cholinesterase. In fact, the acetylcholinesterase (AChE) hydrolyzes only acetylcholine, whereas BchE metabolizes acetylcholine and butyrylcholine (Kluge *et al.*, 2001). For this reason, we used in this work plasma as a source of BchE to study the anticholinesterase potential of *F. communis* L. extracts. In a primary assay, the anti-butyrylcholinesterase activity of the essential oils was tested with different incubation times (10, 15, 25 and 30 min) as presented in Fig. 1.

At 30 min of incubation, all organs (flowers, leaves and stems) of essential oils had the high percentages of

inhibition (58,328 % from the essential oil of flowers, 55,718 % from the essential oil of leaves and 54,718 % from the stems' essential oils). The important inhibition was detected in the leaves' essential oils at the incubation time of 25 min, but the interesting inhibition was that of flowers at 30 min, compared with that of parathion used as a standard drug for the treatment of mild Alzheimer's disease. So for this reason and in a secondary assay, the anti-cholinesterase activity of essential oils of *F. communis* was tested at 30 min of incubation for four concentrations (20, 10, 5, 2.5 and 1.25 mg mL⁻¹). The inhibitory effects of concentrations on BchE are listed in Fig. 2. Flowers' essential oil presents a quite good inhibition (64.623 %) with the concentration of 10 mg mL⁻¹, which was the highest percentage of inhibition.

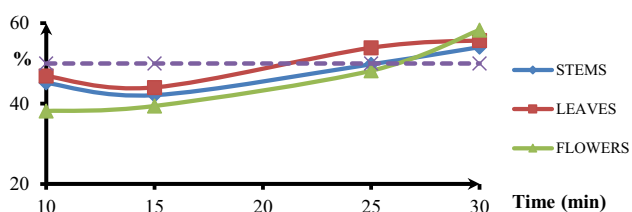


Fig. 1 Variation of inhibition of cholinesterase activity (%) for different organs (flowers, leaves and stems) of *F. communis* for four incubation times at the concentration of 20 mg mL⁻¹

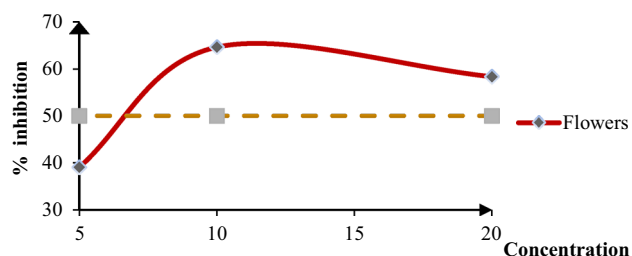
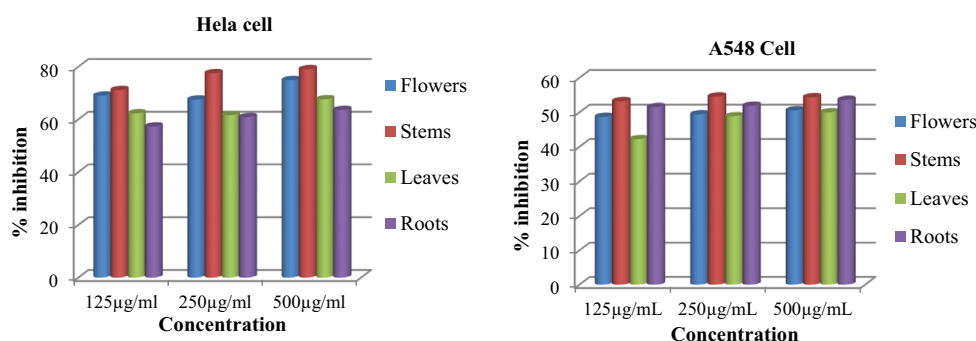


Fig. 2 Variation of cholinesterase inhibition activity (%) for flowers essential oil of *F. communis* in different concentration (mg mL⁻¹)

Fig. 3 Cytotoxicity of essential oils of *F. communis* L. as evaluated by MTT assay and expressed in inhibition percentage (%) at various concentrations



Several essential oils, as well as some of their components, have been reported as having acetylcholinesterase inhibitor ability. However, it is the first time that this property is reported for *F. communis* oils. Sesquiterpenes, the dominant constituents of the essential oils of *F. communis* can partly explain the best activity of these oils, since some studies have revealed that these monoterpenes are potent acetylcholinesterase inhibitors (Smail *et al.*, 2011; Orhan *et al.*, 2008).

Cytotoxic activity

The cytotoxicity of *F. communis* essential oils of different organs (flowers, roots, leaves and stems) was evaluated on A549 lung epithelial carcinoma and Hela cervix cells lines. The cytotoxic effect of the total essential oils was overall important. The inhibition percentage (%) was summarized in Fig. 3.

The activity increased proportionally with the concentration and we noted the most important activity of samples on the two kinds of cells at 500 µg mL⁻¹. All organs present an interesting cytotoxic activity on Hela cells more than on A 549 cells which could suggest a specificity of action toward cells' type.

The best inhibition % on Hela cells was shown with essential oils from stems (79.05 %) and (77.52 %) at the concentrations of 500 and 250 µg mL⁻¹, respectively. The flowers' essential oils present 74.89 % of inhibition % on Hela's cells. These results present the first report on the cytotoxic activity of *F. communis* essential oil.

Conclusions

In conclusion, we have identified the essential oils of different organs (flowers, leaves, stems, and roots), also we have shown that these oils and their associated antioxidants, antimicrobial, anticholinesterase, and cytotoxic possess interested potential to develop a chemopreventive agent against various diseases. These studies have shown a

relationship between results which could be also related to the presence of the same sesquiterpene in the flowers, stems and leaves of essential oils of *F. communis*.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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