

Original Article

Biological Activities and Composition of *Ferulago carduchorum* Essential Oil

Fereshteh Golfakhrabadi¹, Mahnaz Khanavi^{1,2}, Seyed Nasser Ostad³, Soodabeh Saeidnia⁴, Hassan Vatandoost⁵, Mohammad Reza Abai⁵, Mitra Hafizi¹, Fatemeh Yousefbeyk⁶, Yaghoob Razzaghi Rad¹, Ameneh Baghenegadian¹, *Mohammad Reza Shams Ardekani^{1,2}

¹Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

²Department of Traditional Pharmacy, Faculty of Traditional Medicine and Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran

³Department of Toxicology and Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁴Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, Iran

⁵Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

⁶Department of Pharmacognosy, Faculty of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

(Received 18 Aug 2013; accepted 28 June 2014)

Abstract

Background: *Ferulago carduchorum* Boiss and Hausskn belongs to the Apiaceae family. This plant grows in west part of Iran that local people added it to dairy and oil ghee to delay expiration date and give them a pleasant taste. The aim of this study was to investigate the antioxidant, antimicrobial, acetyl cholinesterase inhibition, cytotoxic, larvicidal activities and composition of essential oil of *F. carduchorum*.

Methods: Acetyl cholinesterase (AChE) inhibitory, larvicidal activities and chemical composition of essential oil of *F. carduchorum* were investigated. Besides, antioxidant, antimicrobial and cytotoxic activities of essential oil were tested using DPPH, microdilution method and MTT assay, respectively.

Results: The major components of essential oil were (z)- -ocimene (43.3%), -pinene (18.23%) and bornyl acetate (3.98%). Among 43 identified components, monoterpenes were the most compounds (84.63%). The essential oil had noticeable efficiency against *Candida albicans* (MIC= 2340 µg ml⁻¹) and it was effective against *Anopheles stephensi* with LC₅₀ and LC₉₀ values of 12.78 and 47.43 ppm, respectively. The essential oil could inhibit AChE (IC₅₀= 23.6 µl ml⁻¹). The essential oil showed high cytotoxicity on T47D, HEP-G2 and HT-29 cell lines (IC₅₀< 2 µg ml⁻¹).

Conclusion: The essential oil of *F. carduchorum* collected from west of Iran had anti-*Candida*, larvicidal and cytotoxicity effects and should be further investigated in others *in vitro* and *in vivo* experimental models.

Keywords: *Ferulago carduchorum*, Essential oil, Antimicrobial, Antiacetyl cholinesterase, Larvicidal activity

Introduction

Ferulago carduchorum Boiss and Hausskn (Apiaceae) known as an endemic plant of Iran, grows in west part of Iran (Mozaffarian 2007). In west of Iran, *F. carduchorum* has been traditionally added to dairy and oil ghee to increase hold time and give them a pleasant taste. In the past, this plant was used as natural preservative to delay expiration date of meat, too. Some species of *Ferulago* are

benefit for remedy of digestive pains, hemorrhoid (Sodeifian et al. 2011), disease of spleen, headache, ulcers and snake bites (Demetzos et al. 2000).

Phytochemical studies on *Ferulago* species have led to identification of different coumarins (Ognyanov et al. 1969, Andrianova et al. 1975, Serkerov et al. 1976, Sklyar et al. 1982, De Pascual et al. 1979, Doganca et al. 1991,

Doganca et al. 1992, Ruberto et al. 1994, Jimenez et al. 2000, Khalighi-Sigaroodi et al. 2006). There are also some reports about the acetyl cholinesterase inhibitory (Dall'Acqua et al. 2010), cytotoxic (Rosselli et al. 2009), antimicrobial and antioxidant (Basile et al. 2009) activities of coumarins of *F. campestris*. Furthermore composition of essential oil of 6 different *Ferulago* species (Erdurak et al. 2006, Kilic et al. 2010) and antimicrobial activity of essential oil of *F. bernardii* (Khalighi-Sigaroodi et al. 2005) and *F. campestris* (Cecchini et al. 2010) have been reported later.

Alzheimer's disease (AD) is the most common and important degenerative disease of brain among the elderly and is the fourth leading cause of death in western countries (Shen et al. 2005, Zhou et al. 2008). Reports suggest that AD is caused by reduced synthesis of acetylcholine (Weinstock et al. 1997), so the use of acetyl cholinesterase enzyme inhibitors (AChEIs) could help improve AD (Grutzendler et al. 2001). Traditional AD drugs exhibit side effects and many efforts have been made to achieve a variety of natural AChEIs with less adverse effects (Karimi et al. 2010). Furthermore, a lot of medications for cancer treatment are not effective enough so, researchers are trying to find the most effective medicinal plants to treat cancers (Rahimifard et al. 2009).

Mosquitoes are important vectors in transmission of some human diseases such as malaria, dengue fever, yellow fever and filariasis, which they are substantially among the greatest health problems all over the world (James et al. 1992). Different species of *Anopheles* transmit malaria, filariasis and certain arboviruses (Sedaghat et al. 2005). Malaria is one of the most important diseases in southern Iran (Vatandoost et al. 2011). In previous reports different plants showed toxic effects against public health pests (Hadjiakhoondi et al. 2005, Hadjiakhoondi et al. 2006, Hadji-

akhoondi et al. 2008a, Hadjiakhoondi et al. 2008b, Vatandoost et al. 2008)

The *in vitro* investigation of essential oils is important to estimate their potential to use as an antibiotic or as a drug for AD, cancers, malaria and as a supplement to edible or pharmaceutical products.

The aim of this study was to investigate the antioxidant, antimicrobial, acetyl cholinesterase inhibition, cytotoxic, larvicidal activities and composition of essential oil of *F. carduchorum*.

Materials and Methods

Plant material

The aerial parts of *Ferulago carduchorum* (Apiaceae family) were collected from Manesht mountain of Ilam Province in June 2011. The longitude and latitude of Manesht Mountain are 33°40'60" N and 46°28'0" E. This plant is an endemic plant of Iran, which grows in west part of Iran. The plant was identified and authenticated by Mr Yousef Ajani using flora iranica (Rechinger 1978). The voucher specimen is deposited in herbarium Institute of Medicinal Plants (ACECR), Karaj, Iran (Herbarium number: 1450).

Isolation of the volatile oil

The essential oil was obtained by hydrodistillation using a Clevenger type apparatus (Advanced Technocracy Inc., India) for 4 h according to the European Pharmacopoeia (1975) (Maisonneune 1975). The aerial parts of *Ferulago carduchorum* (Apiaceae family) were dried under shade and powdered. The air-dried parts of *F. carduchorum* (150 g) and 1,000 ml distilled water placed in a round bottom flask (using a fire source from below) connected to a Clevenger-type apparatus. Hydrodistillation is often used to isolate non-water soluble compounds. Hydrodistillation is a method that the plant parts being boiled in water, using a heating source

from below the vessel. In the presence of boiling water volatile compounds are volatilized at a temperature close to 100 °C, at atmospheric pressure. The volatile materials escape in vapor form through some tubes and then are cool. The essential oil is removed from the top of the hydrosol.

1 ml essential oil obtained duration 1 h hydrodistillation. The oil was dried over anhydrous sodium sulphate and kept at 4 °C in the sealed brown vial until required. 1.3 ml essential oil has been obtained from 100 gram dried plant. The oil yield of the plant was determined as 1.3% v/w. The isolation of the volatile oil has been carried out in laboratory of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Gas chromatography mass spectroscopy

Analytical gas chromatography (GC) was carried out using a Shimadzu 15A gas chromatograph with capillary column HP-5ms (60m×0.25mm, ft 0.25mm), carrier gas, He, split ratio 1:25 and using a flame ionization detector (FID). The column temperature was programmed at 60 °C for 3 min and then it was heated to 260 °C at a rate of 5 °C min⁻¹ and the temperature was then kept constant at 260 °C for 15 min. Gas chromatography mass spectroscopy (GC/MS) was carried out on a HP 68900 with a HP 5973 quadruple detector, on capillary column HP-5ms (5% phenyl methyl siloxane) (60m×0.25mm, ft 0.25mm), carrier gas, He, flow rate, 1 ml min⁻¹. The column was held at 60 °C for 3 min and programmed up to 260 °C at the rate of 5 °C min⁻¹, then kept constant at 260 °C for 15 min. The MS was operated at 70 eV ionization energy. Retention indices were calculated using the retention time of n-alkanes that were injected after the oil at the same chromatographic conditions. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oils were identified by com-

parison of their mass spectra and retention indices with Wiley library and those published in the literature (Adams 1995).

DPPH radical scavenging activity

The 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) (Merck, Germany) has a maximum absorption at 517 nm which was used for the investigation of the free radical-scavenging activity of the essential oil (Yokozawa et al. 1998, Khanavi et al. 2009).

Antimicrobial activity

Antimicrobial activities of essential oil of aerial parts of *F. carduchorum* was determined against both Gram-positive (*Staphylococcus aureus* ATCC 6538), Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027) bacteria and a fungal strain (*Candida albicans* ATCC 1023) by microdilution method. Negative control was prepared using dimethylsulphoxide (DMSO), which was solvent used to dissolve the essential oil. Gentamycin was used as positive control against *S. aureus*, *E. coli*, *P. aeruginosa* while, Nystatin was used as positive control against *C. albicans*. Their dilutions ranged from 10 to 0.009 µg/ml concentrations in microtitre plates. The antimicrobial activity of essential oil was assessed by the agar well diffusion method. The plates were incubated at 37 °C for 24 hours for bacteria and 20–25 °C for *C. albicans*. Inhibition was detected by measuring clear zones around the wells in millimeters. Minimum Inhibitory Concentration (MIC) of the essential oil was assessed by broth microdilution method with visible growth observed by using 96 U-shaped wells plates (NCCLS 2006). After 24 h of incubation at 35 °C (bacteria) and 20–25 °C (*C. albicans*), the microdilution plates were tested for the absence or presence of visible growth in comparison with that of the growth in drug-free control well. The endpoint of MIC is the lowest concentration of

the compound at which the test strain does not demonstrate visible growth.

Acetyl cholinesterase (AChE) inhibition

The enzymatic activity was measured using by the method described by Aazza et al. (2011) with minor modifications. 50 µl of buffer 0.1 M (pH 8), 25 µl of essential oil dissolved in dimethyl sulphoxide (DMSO) with different concentrations and 25 µl of 0.22 U/ml of AChE enzyme were mixed. After 15 min incubation at 37 °C, 25 µl of 15 mM acetyl thiocholine iodide (AChI) and 125 µl of 3 mM 5,5'- dithiobis [2-nitrobenzoic acid] (DTNB) were added and the resulting mixture incubated for 30 min at room temperature. Absorbance of the mixture was measured at 405 nm by using a microplate reader (ELX808, BioTek, USA). The inhibitory effect of test compound was calculated by comparing to the negative control: $\% = [(A_0 - A_1) / A_0] * 100$ where A_0 was the absorbance of the blank sample and A_1 was the absorbance of the sample.

The test was repeated three times. The inhibition of enzyme activity was expressed as IC_{50} (the concentration of the sample (µl ml⁻¹), required to inhibit 50% of enzyme), calculated by a linear regression analysis.

Bioassays and larval mortality

Fourth instar larvae of *Anopheles stephensi* Bandar-Abbas strain was exposed to test concentrations of 0.625, 1.25, 2.5, 5, 10, 20, 40 and 80 ppm of essential oil (solvent: Ethanol) for 24 hours according to standard method described by WHO (1981). In briefly 1 ml of appropriate dilution of essential oil with 224 ml of water and 25 larvae in 25 ml water mixed and total volume was 250 ml (Dharmagadda et al. 2005). For control, only 1 ml of ethanol with 224 ml of water and 25 larvae in 25 ml water mixed and total volume was 250 ml. The experiment was repeated four times on different days. The percentage of mortality was reported from

the average for the four replicates after 24 hour exposure period. From the regression line between logarithmic dose and probit mortality, the LC_{50} was determined. The investigation of larvicidal activity has been carried out in the insectarium of Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Cytotoxicity assay

The colon carcinoma (HT-29), breast carcinoma (T47D), hepatocellular carcinoma (HepG2) was obtained from Pasture Institute of Iran, Tehran, Iran. The colon carcinoma HT-29 and T47D (breast carcinoma) cell lines were mentioned as exponentially growing cultures in RPMI 1640 cell culture medium (PAA, Germany), supplemented with 10% fetal bovine serum (FBS: Gibco, USA), for HT-29 cells and 15% FBS for T47D cells. The hepatocellular carcinoma (HepG2) were cultured in Dulbecco's modified Eagle's medium (DMEM, PAA, Germany) supplemented with 10% FBS. The Swiss mouse embryo fibroblast (NIH 3T3) cell line was kept in Dulbecco's modified Eagle's medium (DMEM, PAA, Germany) supplemented with 10% FBS. 100 IU/ml penicillin and 100 µg/ml streptomycin (Roche, Germany) were added to the media. All the cell lines were cultured at 37 °C in air /carbon dioxide (95:5) atmosphere.

Cytotoxic activity was measured using modified MTT assay (Newman DJ 2007). 1×10^4 cells/well were plated in 96-well plates (Nunc, Denmark) and incubated for 24 h before the addition of drugs. After 48 h of incubation in HT-29, NIH/3T3 and MCF-7 cells, 20 µl of MTT (Merck, Germany) reagent (5 mg/ml) in phosphate buffered saline (PBS) was added to each well. The plates were incubated at 37 °C for 4 h. The medium was discharged and the formazan blue, which had been formed in the cells, were dissolved with 100 µl dimethyl sulphoxide (DMSO). After the incubation at 37 °C for

10 min, absorbance at 570 nm at the dissolved solutions was detected using a micro plate reader (Anthos, Austria). The cell viability in MTT assay was calculated as the percentage of control value (Khanavi et al. 2012b). Methotrexate was used as the positive control. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth with 50% ($IC_{50} \pm SD$). All tests and analysis were run in triplicate.

Statistical analysis

In antioxidant and antiacetyl cholinesterase assays, analyses were carried out in triplicate and the data were expressed as mean \pm SD. One-way ANOVA and Tukey post-hoc multicomparison tests were used for the analyses after data normality test. In cytotoxicity assay, IC_{50} (the median growth inhibitory concentration) values were calculated from the IC_{50} of dose-response curve in the sigma plot 11 software. Data representative of three independent experiments with similar results were presented as mean \pm SD. For larvicidal activity was used from MicroProbit software (version 3.0). The percentages of mortality were corrected for the mortality in controls by using Abbott's correction. From the regression line between the logarithmic dose and probit mortality, all the parameters including LC_{50} , LC_{90} , confidence interval (CI) and slope values were determined (Abbott 1925).

Results

Chemical composition of the essential oil

The results of essential oil analysis of *F. carduchorum* were led to identification of 43 compounds (Table 1), represented 92.3% of the total oil. Gas chromatography-Mass diagram of *F. carduchorum* essential oil showed in figure 1. The oil yield of the plant was determined as 1.3% v/w. Major component

of essential oil were identified as (Z)- -ocimene (43.3%), -pinene (18.23%), bornyl acetate (3.98%) and myrcene (3.15%).

Antioxidant and antimicrobial activities

In this research radical scavenging activity of essential oil of *F. carduchorum* was determined and IC_{50} value was calculated as 29.61 μ l. Vitamin E also used as a reference and positive control compound.

In our antimicrobial investigation, the results of Minimum Inhibitory Concentration (MIC) are shown in Table 2. The results of gentamycin and nystatin were used as positive control against bacterial and fungal strain, respectively. The results of positive (gentamycin and nystatin) and negative (DMSO) controls are shown in Table 2.

Acetyl cholinesterase Inhibition Activity

Acetyl cholinesterase inhibition activity of *F. carduchorum* essential oil was studied for the first time. The results showed that the IC_{50} of essential oil was 23.6 μ l ml^{-1} .

Larval mortality

The larvicidal activity of our essential oil and methanol against *Anopheles stephensi* larvae under laboratory conditions are presented in Table 3. Essential oil of *F. carduchorum* was effective against *An. stephensi* with LC_{50} and LC_{90} values of 12.78 and 47.43 ppm, respectively.

Cytotoxicity effect

The effects of *F. carduchorum* essential oil on the proliferative response of the HT-29, HepG2 and T47D cell lines have been analyzed by treating the cells with different concentrations of the essential oil and significant decrease in cell lines proliferation were observed. The results of cytotoxic tests have been shown in Table 4.

Table 1. Chemical composition of essential oil of *Ferulago carduchorum*

No.	Component	RT ^a	RI ^b	Composition (%)
1	-Pinene	10.133	937	18.23
2	Camphene	10.403	946	1.63
3	Verbenene	10.485	958	Trace
4	-Pinene	11.443	971	1.81
5	Myrcene	12.306	989	3.15
6	-Phellandrene	12.697	997	0.4
7	<i>p</i> -Cymene	13.654	1017	0.21
8	<i>o</i> -Cymene	13.500	1020	0.61
9	α -3-Carene	13	1029	0.25
10	-Phellandrene	13.819	1031	2.07
11	(<i>Z</i>)- -Ocimene	14.969	1045	43.3
12	(<i>E</i>)- -Ocimene	15.47	1055	2.71
13	α -Terpinene	16.807	1062	1.82
14	Terpinolene	16.917	1088	0.16
15	Linalool	17.236	1100	0.16
16	1,3,8- <i>para</i> -Menthatriene	18.155	1115	Trace
17	<i>allo</i> -Ocimene	18.744	1129	2.29
18	<i>cis</i> -Verbenol	18.904	1135	0.26
19	neo- <i>allo</i> -Ocimene	18.799	1150	Trace
20	<i>trans</i> -Verbenol	19.162	1156	1.01
21	<i>p</i> -Mentha -1,5-dien-8-ol	20.043	1165	0.39
22	4-Terpineol	20.516	1170	0.13
23	-Terpineol	21.159	1180	0.06
24	Dodecane	22.234	1200	1.02
25	Bornyl acetate	25.446	1277	3.98
26	- Cubebene	33.892	1366	2.65
27	-Ylangene	31.025	1370	0.16
28	-Copaene	29.649	1378	0.36
29	-Bourbonene	29.952	1382	0.43
30	-Cubebene	30.156	1389	0.14
31	-Elemene	30.271	1399	0.14
32	-Gurjunene	36.654	1421	0.32
33	α -Elemene	31.944	1428	0.1
34	Aromadendrene	31.306	1433	0.31
35	-Humulene	32.659	1446	Trace
36	<i>trans</i> - -Farnesene	33.017	1452	Trace
37	Germacrene-D	33.198	1475	Trace
38	Bicyclogermacrene	34.431	1498	0.68
39	- Bisabolene	35.047	1500	0.15
40	α -Cadinene	35.482	1513	0.5
41	Spathulenol	37.424	1568	0.71
42	Salvial-4(14)-en-1-one	37.754	1589	Trace
43	-Cadinol	39.862	1650	Trace
Total				92.3
Monoterpenes hydrocarbons				78.64
Monoterpenes oxygenated				5.99
Sesquiterpenes hydrocarbons				5.94
Sesquiterpenes oxygenated				0.71
Nonterpenoids				1.02

Notes ^a Retention time, ^bRI Retention Index on HP-6890 with reference to n-alkanes injected after the oil at the same chromatographic conditions.

Table 2. Minimum inhibitory concentration (MIC) of *Ferulago carduchorum* essential oil against selected bacteria and *Candida albicans*

Microorganism	MIC ^a	Positive control	Positive control	Negative control
	Essential oil	Gentamycin	Nystatin	DMSO
<i>Staphylococcus aureus</i>	9000	0.62	-	-
<i>Escherichia coli</i>	23000	0.009	-	-
<i>Pseudomonas aeruginosa</i>	18000	2.48	-	-
<i>Candida albicans</i>	2340	-	0.02	-

Note^a: MIC was determined by broth micro dilution method and expressed in $\mu\text{g ml}^{-1}$. - No effect.

Table 3. Probit regression line of *Anopheles stephensi* exposed to different interval concentrations of essential oil of *Ferulago carduchorum*

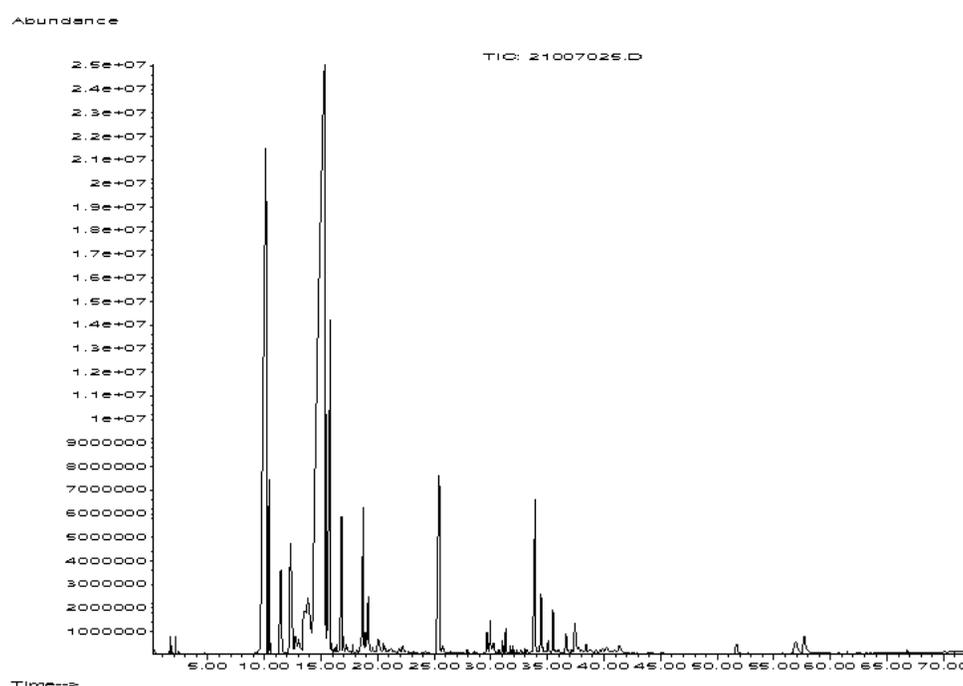
Intercept	Slope \pm SE [*]	LC ₅₀ (ppm)	95% CI	LC ₉₀ (ppm)	95% CI	2	2table (df)	p-value
-2.4906	1.9303 \pm 0.323	12.7818	19.5101– 33.4434	47.4356	89.9890– 357.8734	35.78	22.458 (6)	0.001
						*		

*: No heterogeneity; SE: Standard Error, LC₅₀: Lethal Concentration to cause 50% mortality in population; LC₉₀: Lethal Concentration to cause 90% mortality in population, CI: Confidence Interval, 2 (df) = heterogeneity about the regression line (Degrees Of Freedom).

Table 4. Cytotoxic activity of essential oil of *Ferulago carduchorum*

Cell Lines ^a (MTT assay)	Essential oil	Methotrexate	Doxorubicin
NIH-3T3	0.64 \pm 0.02	0.24 \pm 0.013	0.21 \pm 0.03
HT-29	1.74 \pm 0.14	0.23 \pm 0.02	-
T47D	0.17 \pm 0.01	0.16 \pm 0.09	-
HEP-G2	0.35 \pm 0.02	-	1.04 \pm 0.07

^a: Results are expressed as IC₅₀ values ($\mu\text{g ml}^{-1}$), Key to cell Lines employed: HT-29 (colon carcinoma), T47D (breast carcinoma), HEP-G2 (hepatocellular carcinoma), NIH 3T3 (Swiss embryo fibroblast).

**Fig. 1.** Gas chromatography-Mass diagram of *Ferulago carduchorum* essential oil

Discussion

Among 43 identified components, monoterpenes were the most identified compounds (84.63%) that only 5.99% of them were oxygenated whereas, sesquiterpenes were totally detected about 6.65% with 0.71% oxygenated sesquiterpenes. (Z)- α -ocimene and myrcene as acyclic monoterpene hydrocarbons and β -pinene as a cyclic monoterpene hydrocarbon were identified as main compounds. Moreover, the major oxygen containing monoterpene and sesquiterpene are bornyl acetate (3.98 %) and spathulenol (0.71 %), respectively. Composition of the oil obtained from air-dried aerial parts of *F. carduchorum* from Kerman Province, was found to contain (z)- α -ocimene as the major component (Samiee et al. 2006), which agrees with our research. The amount of (z)- α -ocimene in our investigation (43.3 %) was more than previous study (21.2 %). Moreover *F. carduchorum* from Illam and Kerman Provinces had shown the constituent of β -pinene 18.23 % and 4.8 % respectively. Moreover, other reports showed that in *F. humillis*, *F. trachycarpa* and *F. angulata* (z)- α -ocimene is the main component (Baser et al. 2002, Khanahmadi et al. 2006). The important compound in three species (*F. aucheri* and *F. mughlae* and *F. sandrasica*) was β -pinene. The major components in essential oil of *F. macroseiadia*, *F. sylvatica* and *F. bernardii* were methyl carvacrol, *p*-cymene and 2, 4, 5-trimethyl benzaldehyde, respectively (Baser et al. 2002, Khalighi-Sigaroodi et al. 2005).

Evaluation of MIC of the essential oil showed that sample had antimicrobial effect against both Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and a fungal strain (*Candida albicans*). The essential oil indicated noticeable efficiency against *C. albicans* (MIC= 2340 $\mu\text{g ml}^{-1}$).

The antioxidant assessment of fruits and roots of *F. campestris* were demonstrated

that their IC₅₀ were less than IC₅₀ of essential oil of *F. carduchorum* (Cecchini et al. 2010). Moreover, antimicrobial effect of *F. campestris* was better than *F. carduchorum*, that MIC of roots and fruits essential oils were less than MIC of *F. carduchorum* essential oil (Cecchini et al. 2010). The results of antimicrobial activity of *F. bernardii* essential oil were shown MIC of essential oil against Gram-positive, Gram-negative bacteria and fungal strain were <1000 $\mu\text{g ml}^{-1}$ (Khalighi-Sigaroodi et al. 2005). So, antimicrobial activity of *F. bernardii* is better than *F. carduchorum*. Anti-*candida* effect of *Ferulago capillaris* essential oil was investigated and the results were demonstrated that MIC is less than *F. carduchorum* (Pinto et al. 2013). There have been reports on the AchE inhibitory activity of some bicyclic monoterpenoids including β -pinene and 3-carene (Miyazawa et al. 2005). Hence in our study, AchE inhibitory activity of essential oil could be attributed to its β -pinene.

In a previous study, larvicidal activity of *Eucalyptus camaldulensis* essential oil against *An. stephensi* was investigated and the LC₅₀ and LC₉₀ values were 89.85 and 215.26 ppm, respectively (Sedaghat et al. 2010). In one study, the larvicidal activities of methanolic extracts of some Iranian plants (*Lawsonia inermis*, *Thymus kotschyianus*, *Cedrus deodara* and eight species from *Stachys*) against malaria vector, *An. stephensi* were investigated (Khanavi et al. 2013). These results indicated that essential oil of *F. carduchorum* was more potent than these plants against *An. stephensi*. Compared to another studies on larvicidal activity of essential oils of *Heracleum persicum*, *Foeniculum vulgare*, *Coriandrum sativum* (Sedaghat et al. 2011), *Cymbopogon olivieri* (Hadjiakhoondi et al. 2003) and *Nepeta menthoides* (Khanavi et al. 2012a) it was found that *F. carduchorum* was most effective against *An. stephensi*

(Khanavi et al. 2011). Therefore, the essential oil of *F. carduchorum* can be used in insect control as an alternative for chemical compounds on the environment.

The essential oil of *F. carduchorum* showed a high cytotoxicity on T47D, HEP-G2 and HT-29 cell lines ($IC_{50} < 2 \mu\text{g ml}^{-1}$). The effect of essential oil on T47D cell line was much stronger than HT-29 and HEP-G2. It indicated that the essential oil had potential cytotoxic selectivity on T47D cell line similar to Methotrexate (positive control), whereas, the essential oil had lower cytotoxic effect than Methotrexate on NIH 3T3 (Swiss embryo fibroblast). So, the oil could be safer than this drug. Another study on *Vinca minor* revealed that its cytotoxicity activity on T47D ($IC_{50} = 1.34 \pm 0.29$) and HT-29 ($IC_{50} = 3.63 \pm 1.24$) was less than *F. carduchorum* (Khanavi et al. 2010). In results, this plant has active cytotoxic components.

Conclusion

The essential oil of *F. carduchorum* collected from west of Iran give good biological activity including anti-*Candida*, AchE inhibitory, larvicidal and high cytotoxic effects and should be further investigated in others *in vitro* and *in vivo* experimental models. The determined biological activities of *F. carduchorum* essential oil are important information for its future application in treatment. Furthermore, the determination of antioxidant and antibacterial activity of the essential oil have great importance in preservation of food products and pharmaceutical industries.

Acknowledgements

This research has been supported by Tehran University of Medical Sciences (Grant No: 90-02-33-13855). The authors declare that there is no conflict of interests.

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