



Research Article

Chemical composition of the essential oils and antioxidant capacity evaluation of *Echinophora platyloba* DC. and *Falcaria vulgaris* Bernh. growing in Hamadan province of Iran

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ARTICLE INFO

Keywords:

Falcaria vulgaris
Echinophora platyloba
Antioxidant activity
Essential oil
DPPH
FRAP

ABSTRACT

Background: *Falcaria vulgaris* and *Echinophora platyloba*, two members of Apiaceae family, are native flora of Iran and have numerous ethnobotanical applications. **Objectives:** The present study focuses on the chemical composition of the essential oils and the evaluation of the antioxidant capacity of *F. vulgaris* and *E. platyloba* growing in Hamadan province. **Methods:** Hydrodistilled essential oil from aerial parts of *F. vulgaris* and *E. platyloba* were obtained using clevenger apparatus. The chemical composition of the essential oils was determined using GC-MS analysis. Methanol extracts from aerial parts and seeds of *F. vulgaris* and *E. platyloba* were obtained by Soxhlet extractor. The antioxidant properties of the extracts were determined through use of several antioxidant assays (DPPH, FRAP, β -carotene-linoleic acid and iron-chelating test). **Results:** GC-MS analysis revealed that the major components of *F. vulgaris* essential oil were spathulenol, and palmitic acid. The main components of *E. platyloba* were γ -decalactone and (*E*)-sesquilandulol. The extract from aerial parts of *F. vulgaris* consistently exhibited more antioxidative power than the rest of the extracts, followed by the extracts from aerial parts of *E. platyloba*. The results of DPPH, FRAP and β -carotene-linoleic tests were well correlated with each other, but they were somewhat different from the results of the iron-chelating test. **Conclusion:** The results of this research showed that *F. vulgaris* and *E. platyloba* contain significant antioxidant properties; therefore, their use in food and pharmaceutical industries as preservatives will be fruitful.

1. Introduction

Large amounts of free radicals and reactive oxygen species are produced every day in human body throughout biochemical reactions

that take place in order to maintain hemostasis. Many of them are considered to be the cause of numerous health conditions and lead to diseases like cancers, heart complications, etc [1].

Abbreviations: GC-MS, Gas Chromatography-Mass Spectrometry; FRAP, Ferric Reducing Antioxidant Power; DPPH, 2,2-Diphenyl-1-Picrylhydrazyl

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doi: [10.52547/jmp.21.83.19](https://doi.org/10.52547/jmp.21.83.19)

Received 12 May 2022; Received in revised form 16 July 2022; Accepted 16 July 2022

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Various drugs offer protective effects against oxidative damage, but they come with a variety of negative effects. The employment of plant-based antioxidants obtained from herbal sources has a critical role in reducing the negative side effects of existing drugs [2]. Nowadays, a number of antioxidants have been synthesized from several medicinal herbs [3].

Many plants are rich in free radical scavenging components like terpenoids, phenols, vitamins, etc., all of which possess high antioxidant activity [4].

The family Apiaceae is consisted of 300 genera and has more than 3000 species [5]. Since a great number of its species contain monoterpenes and phenyl components, plants in this family are regarded as potent antioxidants. The genus *Echinophora* belongs to Apiaceae family that solely has ten species which four of them are native to Iran [6]. *Echinophora platyloba* (EP) is the most important of its genus growing in Iran. This species has a large impact on Iranian folk medicine as well as non-medical applications. Recent studies with focus on this species have reported antifungal, antimicrobial and anticancer activities for its extract [7]. There are reports of antioxidant and antifungal properties for the essential oil derived from its aerial parts [8, 9]. Apart from medical applications, this plant is for food seasoning and to halt mold growth in products like pickles and tomato paste among the locals [10]. Also in Azerbaijan province in Iran, It is also used to flavor meat, soup and a variety of dairy products [11].

The genus *Falcaria* from Apiaceae family, has two species that grow in Iran. Those species are *Falcaria vulgaris* and *Falcaria falcarioides* [12]. In folk medicine, *Falcaria vulgaris* (FV) is known by the local name of Ghaziaghi or Paghazeh. It is common for this plant to be

consumed as vegetable in various parts of Iran. Recent studies have suggested numerous therapeutic properties for this specie [13, 14]. In general, this plant is widely used in traditional medicine for purposes such as treatment of heart diseases, skin diseases, contraception, treatment of gastrointestinal disorders and even for analgetic applications [15-17]. A recent study has stated that hydromethanolic extract of FV seems to possess gastroprotective effects on ethanol-induced ulcer in rats [18].

Due to the importance of geographic factors and climate conditions on chemical composition of the plants, in the current study we aimed to evaluate these properties in FV and EP from Hamadan province and compare them to the same species growing elsewhere.

2. Materials and Methods

2.1. Preparation of the Plant Material

Aerial parts of *Falcaria vulgaris* (herbarium code: 111) and *Echinophora platyloba* (herbarium code: 56) were collected in May 2021 from Hamadan province, Iran. The authentication of plant specimens were done in Herbarium of Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. Afterwards they were dried and stored at room conditions.

2.2. Preparation of the extract

100 g of the dried parts of each plant was soaked in methanol for 3 days. The solvent was evaporated using rotary vacuum evaporator. This procedure was repeated 3 times to attain the plant extract. Notably, the efficiency of the extraction process for aerial parts and seeds of FV and aerial parts of EP were 12.02 %, 11.76 % and 5.98 %, respectively.

2.3. Preparation of the essential oil

The essential oils of the plants were prepared by hydrodistillation using a cleverger-type apparatus, then isolated and kept in sealed dark glass vials at 4 °C until use. Briefly, 200 g of the aerial parts of FV and EP yielded 0.5 ml and 0.8 ml of their essential oils, respectively.

2.4. GC-MS Analysis

The essential oils were analyzed using a ThermoQuest-Finnigan TRACE MS gas chromatograph-mass spectrometer (GC-MS) fitted with a fused methyl silicon DB-5 column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1.1 ml/min. The column temperature was kept two min at 60 °C, increased to 250 °C at a rate of 5 °C/min increase, and finally 2 min at 245 °C. The injector temperature was 250 °C and split ratio was adjusted at 1/100, 1/10. The injection volume was 0.2 µl. Mass spectrometer condition was as follow: ionized potential 70 eV and source temperature 200 °C. Constituents were identified by comparison of their Retention Index relative to C₅-C₂₄ n-alkanes by comparison of the RI, provided in the literature, by comparison of the mass spectra with those recorded by the MAINLIB and Willey.

2.5. DPPH assay

Free radical scavenging capacity of the extracts of FV and EP was determined based on a method described by Kamkar et al. with minor modification [19]. In this assay, antioxidant or electron donation ability of the plant is determined by bleaching of the purple-colored methanol solution of DPPH. Briefly, a 750 µl of various concentrations of plant extract and essential oil in methanol (4000-31.5 µg/ml) was added to 300 µl of 0.3 mM DPPH radicals in methanol at a ratio of 5:2, respectively. The

mixture was let to stand at room conditions for 30 minutes. The absorbance of the solution was measured against a blank at 518 nm. All measurements were performed in triplicate.

Antioxidant activity (%) was achieved using the following formula:

$$AA\% = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] \times 100$$

Where Abs_{Control} is the absorbance of the control reaction (containing all reagents apart for the test compound) and Abs_{Sample} is the absorbance of the test compound. ascorbic acid and galic acid were used as positive standards. Extract concentrations providing 50 % inhibition (IC₅₀) were figured from the graph plotted of inhibition percentage against their concentration.

2.6. β-Carotene-linoleic acid assay

In this study β-carotene-linoleic acid assay was performed based on a study by Gursoy et al. (2006) with a few modifications [20]. A stock solution of β-carotene-linoleic acid was prepared as follows:

0.5 mg of β-carotene was dissolved in 1 ml of chloroform. Later on, 25 µl of linoleic acid and 200 mg of Tween 40 was added to the mixture. Chloroform was entirely evaporated using a rotary vacuum evaporator. Afterwards, 100 ml of deionized water was added and the mixture underwent a vigorous shaking in order to form an emulsion. 1.5 ml of this reaction mixture was placed in the test microtubes along with 250 µl of various concentrations (0.625-8 mg/ml) of the extracts of FV and EP in methanol; The absorbance was immediately read at 490 nm at T zero. Then the system was incubated up to 2 hours at 50 °C and the absorbance was measured again. The same procedure was repeated for ascorbic acid and gallic acid as positive controls along with a

blank sample. Antioxidative activities of the extracts were put to comparison to those of ascorbic acid and gallic acid at 0.5 mg/ml and blank. All measurements were performed in triplicate.

The antioxidant activity (%) were calculated as following:

$$AA\% = [1 - (Ab_{Sample(2h)} - Ab_{Sample(T\ zero)}) / (Ab_{Control(2h)} - Ab_{Control(T\ zero)})] \times 100$$

Where $Ab_{Control(2h)}$ and $Ab_{Sample(2h)}$ are the absorbance of control reaction (which contains all reagents apart for the test compound) and test compound at 2 hours, and $Ab_{Control(T\ zero)}$ and $Ab_{Sample(T\ zero)}$ are their absorbance at the start of the preparation.

2.7. Ferric Reducing Antioxidant Power (FRAP) Assay

In this study the ferric reducing power was determined by the means of a modified version of the FRAP assay described by Szöllösi et al. [21].

The working FRAP reagent was freshly prepared by mixing 25 ml of 300 mM acetate buffer (pH 3.6), 2.5 ml of 10m M/L TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40mM/L HCl and 2.5 ml of 20 mM $FeCl_3$. A standard curve was generated using various concentrations (100-0.625 mM) of $FeSO_4 \times 7H_2O$. All solutions were made daily and used the through the course of that same day. 50 μ l of each concentration (0.062-8 mg/ml) of the extracts were added to 1.5 ml of FRAP reagent. The reaction mixture was incubated for 30 minutes at 37 °C in a water bath. The absorbance was afterwards read at 593 nm against a blank which contained 50 μ l of methanol. Ascorbic acid and gallic acid were employed as positive controls. Relative activities were derived from standard curve of $FeSO_4 \times 7H_2O$ standard solutions, then FRAP values were expressed as mmol Fe^{2+} per

1 g of plant extract. All measurements were performed in triplicate.

2.8. Determination of iron-chelating activity

The evaluation of the iron-chelating activity of the extracts relies on the ability to intervene in formation of Ferrozine- Fe^{2+} complex. We followed a protocol described by Chew et al. with minor modifications [22].

Briefly, a mixture of 0.2 ml of each extract concentrations (8-0.625 mg/ml), 0.2 ml of 0.10 mM $FeSO_4$ and 0.4 ml of 0.25 mM ferrozine were incubated at room conditions for 10 minutes. Afterwards the absorbance of the mixture was determined at 562 nm. Accompanying the test compounds, a blank solution was prepared for each measurement through replacing ferrozine and $FeSO_4$ with water to correct the background absorbance. All measurements were performed in triplicate.

The iron-chelating ability (A) was calculated using the following equation:

$$A (\%) = [1 - (Ab_{Sample}/Ab_{Control})] \times 100$$

Where $Ab_{control}$ is the absorbance of control reaction (which contained all reagents apart for the test compound) and Ab_{sample} is the absorbance in presence of extract/standard. Disodium salt of EDTA and ascorbic acid were employed as positive controls. IC_{50} value, which defines as the extract or positive control concentration required to attain 50 (%) chelating activity, was calculated from the plot of chelating activity percentages against extract concentration.

3. Results

3.1. Chemical Compositions of the Essential Oils

Table 1 shows the chemical compositions of the essential oil of *Echinophora platyloba*. A total of 25 separate chemical constituents were

identified. γ -decalactone (21.3 %), (*E*)-sesquilandulol (15.81 %), palmitic acid (11.06 %), 9,10-dehydroisolongifolene (9.59 %), and *trans*- β -ocimene (5.44 %) followed by *n*-docosane (4.27 %) were the major components (Fig. 1).

As for *Falcaria vulgaris*, 24 separate chemical compounds were detected (Table 2). Spathulenol (53.37 %), palmitic acid (14.63 %), thymol (6.68 %), hexahydrofarnesyl acetone (6.11 %) and carvacrol (2.76 %) were the main constituents (Fig. 2).

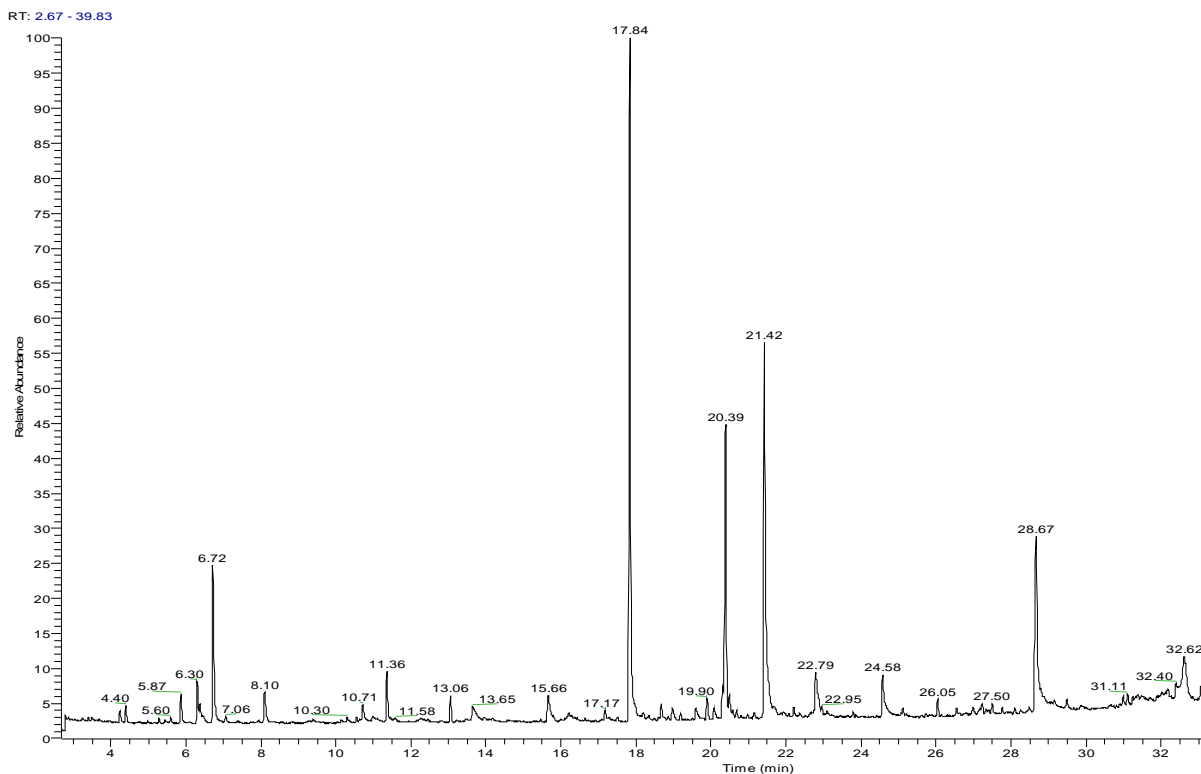


Fig. 1. GC chromatogram of the essential oil from aerial parts of *Echinophora platyloba*

Table 1. Chemical composition of the essential oil from aerial parts of *Echinophora platyloba*

No.	RT	RI	Compound	Area %
1	4.24	928	α -Thujene	0.39
2	4.4	937	α -Pinene	0.49
3	5.87	1012	α -Phellandrene	0.96
4	6.3	1030	<i>p</i> -Cymene	1.36
5	6.38	1034	Limonene	0.65
6	6.72	1048	<i>trans</i> - β -Ocimene	5.44
7	8.1	1105	6-Camphenone	1.27
8	10.71	1205	Terpineol	0.59
9	11.36	1229	2-Ethylhexyl acrylate	1.52
10	13.06	1291	Dihydroedulan II	0.91

Table 1. Chemical composition of the essential oil from aerial parts of *Echinophora platyloba* (Continued)

No.	RT	RI	Compound	Area %
11	15.66	1390	4-Methyl-2-phenyl-2-pentenal	1.60
12	17.84	1476	γ-Decalactone	21.30
13	18.68	1510	2,4-Di-tert-butylphenol	0.49
14	19.9	1561	Nerolidol	0.68
15	20.31	1578	cis-3-Hexenyl benzoate	0.79
16	20.39	1582	9,10-Dehydroisolongifolene	9.59
17	20.49	1586	Caryophyllene oxide	0.89
18	21.42	1626	(E)-Sesquilandulol	15.81
19	22.79	1687	γ -Dodecalactone	1.45
20	22.85	1689	α -Bisabolol	0.60
21	24.58	1771	Tetradecanoic acid	1.92
22	26.05	1842	Hexahydrofarnesyl acetone	0.66
23	28.67	1971	Palmitic acid	11.06
24	32.4	2172	Stearic acid	0.52
25	32.62	2184	n-Docosane	4.27
Total Identified				85.21

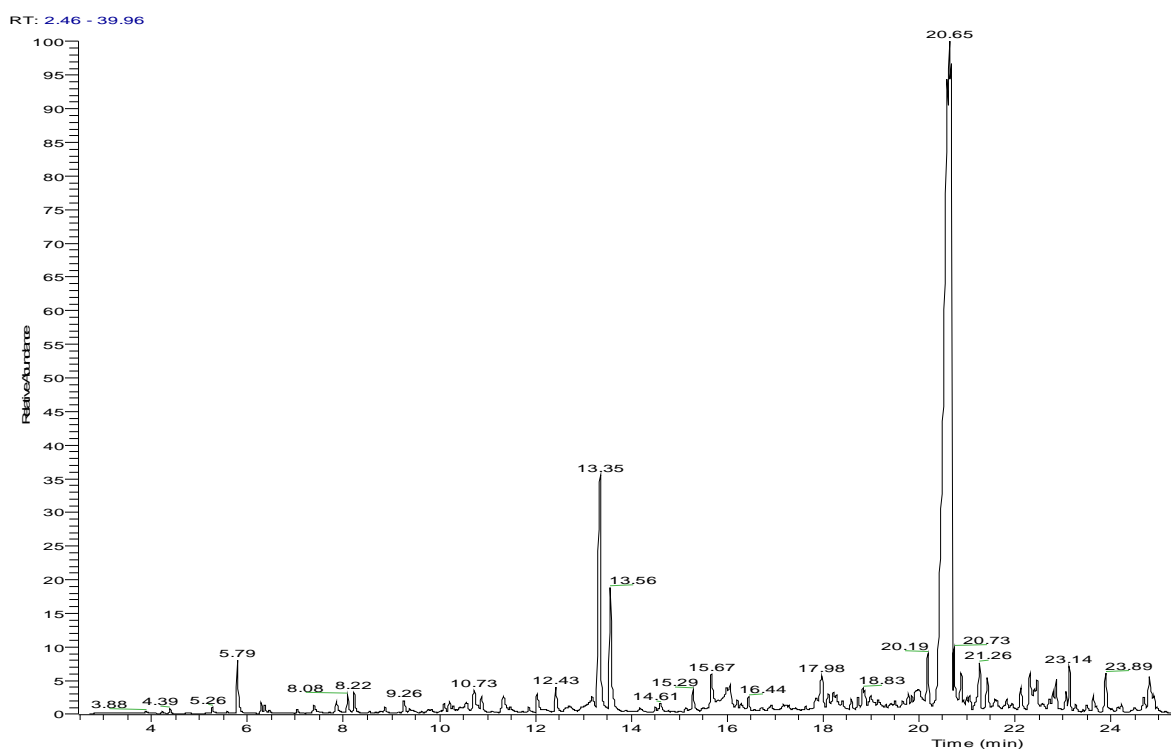
**Fig. 2.** GC chromatogram of the essential oil from aerial parts of *Falcaria vulgaris*

Table 2. Chemical composition of the essential oil from aerial parts of *Falcaria vulgaris*

No.	RT	RI	Compound	Area GCMS %
1	5.79	1009	Octanal	0.91
2	10.73	1206	Myrtenol	0.67
3	12.03	1253	Cuminaldehyde	0.42
4	12.43	1268	(E)-2-Decenal	0.47
5	13.35	1302	Thymol	6.68
6	13.56	1310	Carvacrol	2.76
7	15.29	1376	α -Copaene	0.48
8	15.67	1390	4-Methyl-2-phenyl-2-pentenol	1.00
9	17.98	1478	trans- β -Ionone	1.07
10	18.22	1487	β -Guaiene	0.37
11	18.83	1516	2,4-Di-tert-butylphenol	0.42
12	20.19	1573	Salvial-4(14)-en-1-one	1.09
13	20.65	1592	Spatulenol	53.37
14	20.73	1596	cis- α -Copaene-8-ol	0.46
15	20.89	1627	Isospathulenol	0.73
16	21.26	1657	9-Aristolene- α -ol	0.55
17	21.43	1665	Cadinol	0.95
18	22.12	1673	Turmerone	0.53
19	22.31	1690	Aromadendrene oxide II	0.55
20	22.47	1702	Germacrene-4(15),5,10(14)-trien-1 α -ol	0.74
21	22.86	1847	Hexahydrofarnesyl acetone	6.11
22	23.14	2004	Palmitic acid	14.63
23	23.89	2157	Linoleic acid	1.13
24	24.8	2164	Ethyl linoleate	0.95
Total Identified				97.04

3.2. Free radical-scavenging activity in DPPH assay

The concentration of antioxidant required to decrease the initial DPPH concentration by 50 % (IC₅₀) is a parameter most commonly employed to determine the antioxidant activity [23].

IC₅₀ values of the extracts from aerial parts and seeds of FV as well as EP are illustrated in

Table 3. Our findings revealed that increase in extract concentrations will lead in a rise in free radical-scavenging activity of every extracts. Activity of the methanol extract of aerial parts of FV was higher than the other extracts (P < 0.05) but still, It wasn't comparable to gallic acid (IC₅₀ = 2.181 ± 0.375 µg/ml) and ascorbic acid (IC₅₀ = 8.82 ± 0.589 µg/ml) as positive controls (Fig. 3).

3.3. Inhibitory effect of the extracts on lipid peroxidation in β -carotene-linoleic acid assay

Inhibition on lipid peroxidation in response to the extracts of EP and FV is demonstrated in Fig. 4. The methanol extract from aerial parts and seeds of FV effectively inhibited the oxidation of linoleic acid as much as $65.82 \pm 0.47\%$ and $56.21 \pm 0.26\%$, respectively (Table 4). The extract from EP was as effective as $59.12 \pm 0.42\%$ at the highest concentration. The

methanol extract from aerial parts of FV exhibited significantly more activity than other extracts at all concentrations ($P < 0.05$). Notably, the antioxidant capacity of the extract from aerial parts of FV at 8 mg/ml was comparable to gallic acid at 0.5 mg/ml. At the same concentrations, neither extract nor essential oil were comparable to positive controls.

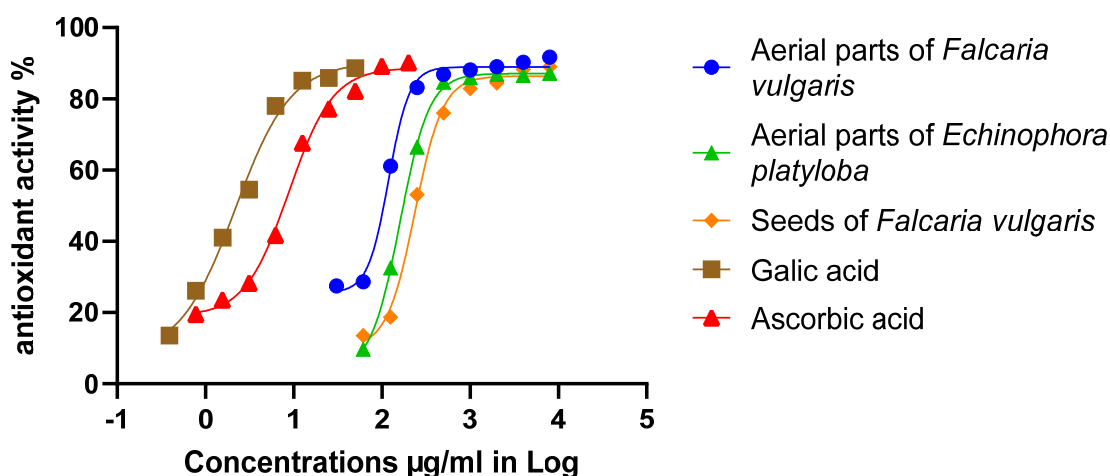


Fig. 3. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl free radical

Table 3. Scavenging effect (%) on 1,1-diphenyl-2-picrylhydrazyl

Sample	DPPH (IC ₅₀ µg/ml)
Aerial parts of <i>Falcaria vulgaris</i>	119.05 ± 5.45
Seeds of <i>Falcaria vulgaris</i>	236.9 ± 15.6
Aerial parts of <i>Echinophora platyloba</i>	160.45 ± 5.75
Galic acid	2.181 ± 0.375
Ascorbic acid	8.825 ± 0.589

Values expressed are means ± SD of three parallel measurements.

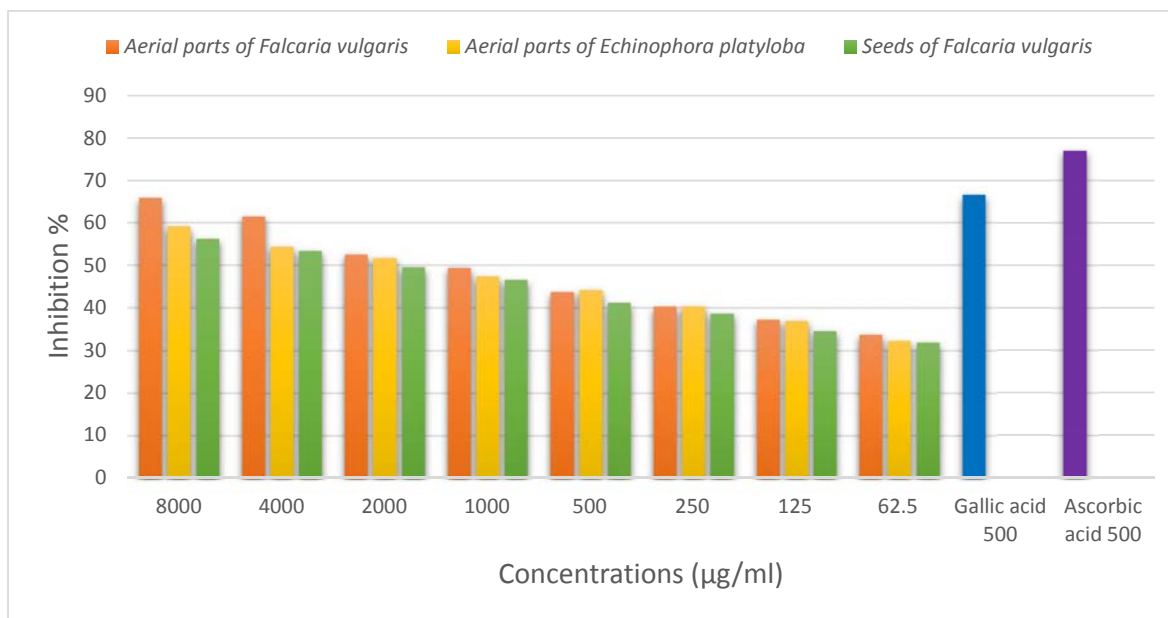
Table 4. Antioxidant activity (%) of extracts in β -carotene-linoleic acid assay

Sample	Concentration (µg/ml)				
	8000	4000	2000	1000	500
Aerial parts of <i>Falcaria vulgaris</i>	65.82 ± 0.47 ^a	61.47 ± 0.32	52.49 ± 0.46	49.38 ± 0.58	43.61 ± 0.41
seeds of <i>Falcaria vulgaris</i>	56.21 ± 0.26	53.38 ± 0.33	49.47 ± 0.63	46.51 ± 0.37	41.11 ± 0.34
Aerial parts of <i>Echinophora platyloba</i>	59.12 ± 0.42	54.33 ± 0.36	51.72 ± 0.24	47.34 ± 0.32	44.19 ± 0.29
Galic acid	-	-	-	-	66.52 ± 0.84
Ascorbic acid	-	-	-	-	76.92 ± 1.26

Table 4. Antioxidant activity (%) of extracts in β -carotene-linoleic acid assay (Continued)

Sample	Concentration ($\mu\text{g/ml}$)		
	250	125	62.5
Aerial parts of <i>Falcaria vulgaris</i>	40.25 ± 0.22	37.17 ± 0.39	33.62 ± 0.18
seeds of <i>Falcaria vulgaris</i>	38.57 ± 0.41	36.91 ± 0.12	32.14 ± 0.17
Aerial parts of <i>Echinophora platyloba</i>	40.34 ± 0.48	36.91 ± 0.23	32.14 ± 0.36
Gallic acid	-	-	-
Ascorbic acid	-	-	-

^aValues expressed are means \pm SD of three parallel measurements.

**Fig. 4.** Antioxidant activity of *F. vulgaris* and *E. platyloba* defined as inhibition percentage through β -carotene-linoleic acid assay

3.4. Ferric Reducing Antioxidant Power Assay

Calibration curve for FeSO_4 standard solution is illustrated in Fig. 5. The results of FRAP assay for the extracts of EP and FV are presented in Table 5. The trend for the ferric ion reducing activity did not vary significantly from DPPH assay done in this study. Same as the DPPH assay, the extract from aerial parts of FV expressed the most activity, followed by the extract from EP. Although the differences in reducing power of all the extracts were not that grave, but still the extract from seeds of FV was proven to possess lesser power than the rest. Notably in terms of reducing power, leaves of

FV have more than 2 times greater activity compared to its seeds ($P < 0.05$). nevertheless even the activity of extract from aerial parts of FV was still modest when compared to ascorbic acid and gallic acid as positive controls. As it can be observed from Table 5, 1 g of the extract of aerial parts, seeds of FV and EP possessed an equivalent of 41.11, 17.62 and 29.51 mmol Fe^{2+} ions, respectively. Since 100 g of dried powder of the mentioned plants provided 12.02, 11.76, and 5.98 g of extract, therefore there are an equivalent of 4.94, 2.07 and 1.76 mol Fe^{2+} (832.19 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) in 1 kg of their plant materials, respectively. Therefore in general,

although EP has more reducing activity than the seeds of FV, but since provides less yield of extract from its plant material, the same quantity of its plant material has lower amounts of Fe^{2+} ions compared to the seeds of FV.

3.5. Iron-chelating Assay

Iron-chelating activity of the tested extracts in descending order was as following: EP extract > FV seed extract > FV aerial parts extract (Fig. 6). In general iron-chelating activity elevated with increase in extract concentrations. The *E. platyloba* had the highest activity that was significantly different from all of the other fractions ($P < 0.05$). IC_{50} values of each extract are illustrated in Table 6. Apart for

the EP extract (IC_{50} : 1.068 ± 0.074 mg/ml), Iron-chelating of the other fractions were modest in comparison to EDTA (IC_{50} : 0.021 ± 0.001 mg/ml) as positive control ($P < 0.05$). Since ascorbic acid expresses a good antioxidative potential in DPPH, β -carotene bleaching and FRAP assays, we decided to assess its chelating activity as well, to put it in comparison with EDTA and other extract. IC_{50} value of ascorbic acid was found to be 0.387 ± 0.021 mg/ml which was almost twice as powerful as the extract from EP.

Our findings suggest that the chelating characteristics of EP extract on Fe^{2+} ions, could to some extent, provide a protective effect against oxidative damage.

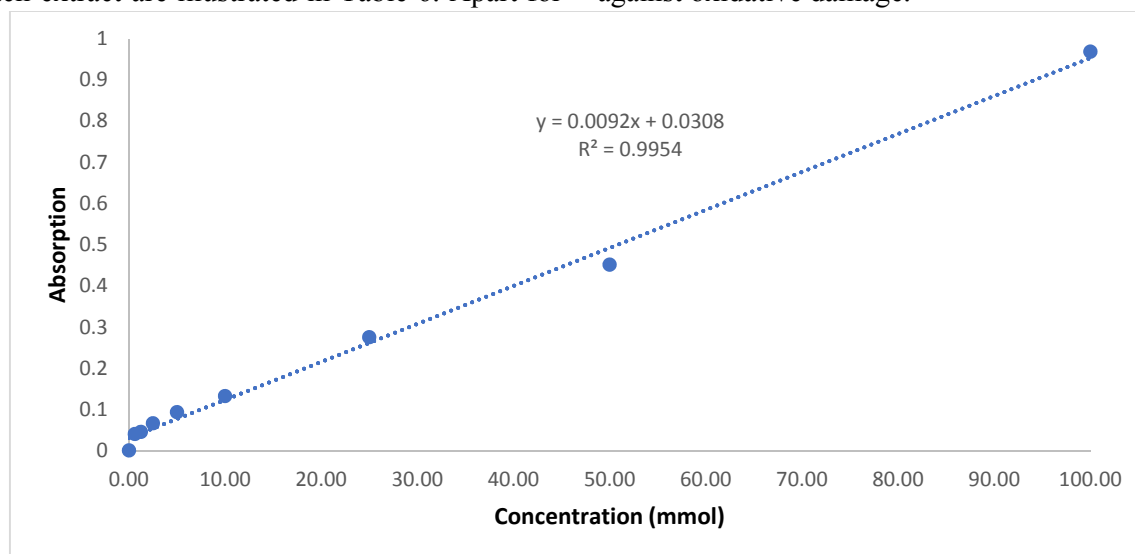


Fig. 5. Linearity of FRAP (dose-response line) for $\text{FeSO}_4 \times 7\text{H}_2\text{O}$ standard solutions

Table 5. Antioxidant activity of extracts using FRAP assay

Sample	FRAP (mM Fe^{2+} /g)
Aerial parts of <i>Falcaria vulgaris</i>	41.11 ± 7.39
seeds of <i>Falcaria vulgaris</i>	17.62 ± 4.01
Aerial parts of <i>Echinophora platyloba</i>	29.51 ± 7.9
Ascorbic acid	195.26 ± 4.69
Galic acid	672.86 ± 21.25

Values expressed are means \pm SD of three parallel measurements.

Table 6. IC₅₀ values of each fraction. Each value is mean \pm standard deviation of three replicate analyses

Fractions	Fe ²⁺ chelating activity (IC ₅₀ mg/ml)
Aerial parts of <i>Falcaria vulgaris</i>	3.19 \pm 0.198
seeds of <i>Falcaria vulgaris</i>	2.34 \pm 0.136
Aerial parts of <i>Echinophora platyloba</i>	1.07 \pm 0.074
EDTA	0.02 \pm 0.001
Ascorbic acid	0.39 \pm 0.021

Values expressed are means \pm SD of three parallel measurements.

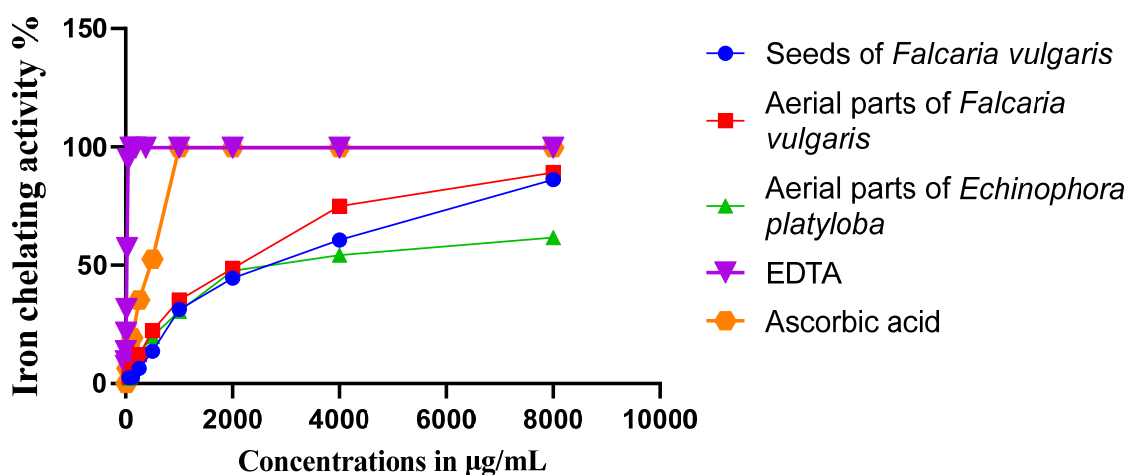


Fig. 6. Iron-chelating activity (%) of the extracts from aerial parts and seeds of *F. vulgaris* and *E. platyloba* compared with EDTA and ascorbic acid. Data are mean \pm SD (n = 3)

4. Discussion

4.1. GC-MS

Among numerous studies that had evaluated chemical composition of the essential oil from various locations in Iran, β -ocimene (28 % -68 %) is reported to be the principal component in *Echinophora platyloba* essential oil [8, 11, 24, 25]. Amounts of this compound was lower in our sample; this can emphasize the importance of the precise location and the type of climate which this plant is harvested from on chemical composition. Moreover, other studies suggested that thymol (27.2 %) and carvacrol (7.2 %) are also considered as main components of this species, where no trace of these two compounds were found in this essential oil.

Based on previous research, there are four chemotypes reported for *E. platyloba* essential oil to date: 1) β -ocimene, δ -3-carene, 2) thymol, trans-ocimene, carvacrol, 3) asarone, anethole, eugenol, 4) β -ocimene, α -caryophyllene. Our results show that the essential oil from *E. platyloba* growing in Hamadan province falls into the last category, containing β -ocimene and Caryophyllene in higher amounts comparing to the rest.

Our results were in accord with the ones from a study done by Khanahmadi et al. on *F. vulgaris* growing in Dalahoo region on Kermanshah province [26]. They reported an amount of spathulenol (27.8 %) and carvacrol (20.93 %) in their essential oil sample. Although

carvacrol was detected in lower quantities in our plant, but the amounts of Spanthulenol were almost as twice as the sample from Dalahoo region. Once again this demonstrate the difference that is made in chemical composition of the plants solely by the environment in which they grow.

Falcaria falcariodes is also grows in Iran. A study by Masoudi et al revealed that Germacrene B (67.9 %) was the principal component of this plant among the other 24 chemical compounds detected in the essential oil [27]. Recent studies have shown that Spanthulenol has the potential to be a good candidate for help to treat drug resistance in cancer treatments [28].

4.2. DPPH assay

Our results from this assay show that the extract from aerial parts of *F. vulgaris* was the most powerful in terms of antioxidant activity with an IC_{50} of $119.05 \pm 5.45 \mu\text{g/ml}$, closely followed by the extract of *E. platyloba* (IC_{50} : $160.45 \pm 5.75 \mu\text{g/ml}$). The extract form seeds of *F. vulgaris* possessed the least activity among others (IC_{50} : $236.9 \pm 15.6 \mu\text{g/ml}$). Although none of the mentioned extracts were comparable with the positive controls, their activities were still impressive while they were only crude methanol extracts of the plants. Enrichment of the extracts and isolating their different fractions on the basis of polarity, could prove to be beneficial for achieving more antioxidant power.

In a similar study, Gholivand et al. investigated the chemical composition and in vitro antioxidant activities of essential oil and methanol extracts of *E. platyloba* [29]. For determination of the antioxidant activity of the plant, they prepared polar and non-polar subfractions of the methanol extract from *E. platyloba* and subjected both of these extracts

to DPPH, as well as the measurement of total phenol content. They reported that polar and non-polar subfractions of the methanol extract from *E. platyloba* have an IC_{50} of $71.2 \pm 1.11 \text{ mg/ml}$ and $331.4 \pm 4.52 \text{ mg/ml}$, respectively. It was noteworthy that these polar and non-polar extracts possessed $67.5 \pm 0.48 \text{ mg/ml}$ and 35.3 ± 0.12 of total phenols. It can easily be noticed that the amount of total phenols correlates well with the antioxidative activity as the polar extract with higher phenol content has almost 3 times more antioxidative activity than its non-polar counter part.

A similar study by Zangeneh et al. reported an IC_{50} of $392 \pm 1.15 \mu\text{g/ml}$ for the extract from aerial parts of *F. vulgaris* [30]. When compared to our work, the results show that *F. vulgaris* from Hamadan province has notably more potent antioxidant characteristics than the same species growing in Kermanshah province.

4.3. β -Carotene-linoleic acid assay

Antioxidant capacity of aqueous and ethanolic extract of *E. platyloba* was measured in a study done by Sharafati-chalesshtori et al. by the means of β -carotene-linoleic acid assay. These authors stated that at $500 \mu\text{g/ml}$, aqueous and ethanolic extracts of the plant had $45.5 \pm 2.1 \%$ and $42 \pm 1.8 \%$ antioxidant capacity in β -carotene –linoleic acid assay, respectively. They compared their results with a sample of BHT as positive standard. In their experiment BHT was proven to possess $89.37 \pm 3.4 \%$ antioxidant capacity [31]. Comparing to our work, where 44.19% antioxidant capacity was noted for $500 \mu\text{g/ml}$ concentration, we realize that *E. platyloba* from Charmahal va Bakhtiari province has the same power as the plant from Hamadan province.

Similarly, Gholivand et al. used the same method to evaluate the antioxidant activity of

polar and non-polar subfractions of methanol extracts from *E. platyloba* [29]. They discovered that non-polar extract possessed more antioxidative effects with 60.7 ± 0.66 % relative antioxidative activity (RAA), opposed to the polar extract with an activity of 31.6 ± 0.91 %.

4.4. FRAP assay

A study by Zenjin et al. reported that *F. vulgaris* from Turkey possess 62.23 ± 1.31 mg of Trolox Equivalent per 1 gram of the extract [32]. Another research by Ghasemi Pirbalouti et al. showed the extract from *E. platyloba* has an EC_{50} value of 1.15 ± 0.078 mg/ml in FRAP assay [9]. Since both of these studies employed different methods in their measurement of reducing power, no direct comparison could be made between their results and our data but nevertheless it provides context for future research to conduct their experiments.

4.5. Iron-chelating assay

Similar to our work, a study was done by Zengin et al. which focused on the Phytochemical characterization of five plants from Apiaceae family. They took liberty of using DPPH, FRAP, CUPRAC, ABTS and iron-chelating assays for the measurement of the antioxidative activity. They reported that *F. vulgaris* possess a chelating power of 23.37 ± 1.18 mg EDTA equivalent per 1 gram of the extract. Notably among these five species, *F. vulgaris* was the least powerful in terms of metal-chelating power [32].

The antioxidant activity and total phenol content of six *Mentha* species from the *Lamiaceae* family were investigated by Benabdallah et al [33]. The select *Mentha* species were: *aquatica*, *arvensis*, *piperita*, *pulegium*, *rotundifolia* and *villosa*. They employed DPPH assay, β -carotene bleaching

and iron chelating test to determine the antioxidant activity of the foresaid plants. The results from iron chelating tests revealed that *M. aquatica* has the highest chelating activity with an IC_{50} of 0.70 ± 0.80 mg/ml. The IC_{50} value of other species varied from 0.80 ± 0.70 to 1.50 ± 0.90 mg/ml. Comparing these results to our data, we observe *E. platyloba* has an intermediate chelating power among *Mentha* species from Apiaceae family and *F. vulgaris* is not a powerful iron-chelator as any of the tested plants.

In another related study, Dorman et al. investigated the antioxidant activity of multiple *Lamiaceae* family plants native to Turkey [34]. *Thymbra spicata* L., *Satureja cuneifolia*, *Coridothymus capitatus* L., *Origanum syriacum* L. and *Origanum onites* L. were among others. They employed DPPH assay, FRAP assay and iron chelating activity test along with non-site and site-specific hydroxyl radical scavenging assays to measure the antioxidant activity of the quoted plants. The data from iron chelating test stated that IC_{50} of studied plants ranged from 0.93 to 1.42 mg/ml which belonged to *O. syriacum* and *S. cuneifolia*, respectively. Therefore, we acknowledged that *E. platyloba* had more chelating power than all of those plants with an exception of *O. syriacum*. But the extract from both seeds and aerial parts of *F. vulgaris* failed to compete with any of the experimented plants in terms of iron-chelating power.

Considering these two research, we may conclude that in general plants in *Lamiaceae* family are prone to show more chelating power than the ones from Apiaceae family. Although *E. platyloba* was more potent than *F. vulgaris* and some of the plants from *Lamiaceae* family, but it still ranked among the less powerful iron-chelators plants of this family.

5. Conclusion

The chemical composition of the essential oils showed that the major components of *F. vulgaris* essential oil are Spathulenol, Thymol and Carvacrol, whereas the main constituents of *E. platyloba* essential oil are Decalactone and Sesquilandulol. There was a correlation in all of the performed assays regarding the antioxidative investigation. Apart for the iron-chelating test, the extract from aerial parts of *F. vulgaris* was persistently found to be more powerful than the rest of the samples, followed by the extract from aerial parts of *E. platyloba* and the least antioxidative power was belonged to the extract from seeds of *F. vulgaris*. In iron-chelating test the results were different, where *E. platyloba* had the most chelating activity followed by extract of seeds and aerial parts of *F. vulgaris*.

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Author contributions

S.M. designed the experiments, supervised the work, interpreted the data and wrote the article. M.S. carried out the experimental bench work and analyzed. Both authors approved the final version of the manuscript.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

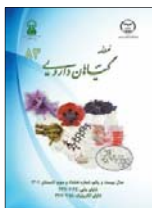
The financial support from Vice chancellor of Research and Technology, Hamadan University of Medical Sciences for the conduct of this work is gratefully acknowledged (Grant Number: 140002211124).

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How to cite this article: Soleimani Shadvar M, Moradkhani SH. Chemical composition of the essential oils and antioxidant capacity evaluation of *Echinophora platyloba* DC. and *Falcaria vulgaris* Bernh. growing in Hamadan province of Iran. **Journal of Medicinal Plants** 2022; 21(83): 19-34. doi: 10.52547/jmp.21.83.19



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مقاله تحقیقاتی

بررسی ترکیبات شیمیایی اسانس و ارزیابی اثرات آنتی اکسیدانی گیاهان پاغازه و خوشاریزه رویش یافته در استان همدان

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اطلاعات مقاله	چکیده
گل وازگان:	مقدمه: گیاهان پاغازه (<i>Falcaria vulgaris</i>) و خوشاریزه (<i>Echinophora platyloba</i>)، دو عضو از خانواده
پاغازه	چتریان (<i>Apiaceae</i>) در میان فلور طبیعی ایران بوده و حاوی خواص فراوانی هستند. هدف: هدف مطالعه ی حاضر
خوشاریزه	بررسی ترکیبات شیمیایی اسانس و اثرات آنتی اکسیدانی عصاره ی گیاهان پاغازه و خوشاریزه می باشد. روش بررسی:
فعالیت آنتی اکسیدانی	اسانس گیاهان با استفاده از روش تقطیر با آب و دستگاه کلونجر به دست آمد و ترکیبات شیمیایی آن توسط
اسانس	کروماتوگرافی گازی متصل به طیف سنج جرمی بررسی گردید. عصاره های متانولی این دو گیاه نیز به روش استخراج
DPPH	با سوکسله تهیه شدند و تست های آنتی اکسیدانی DPPH، FRAP، بتاکاروتن-لینولئیک اسید و شلاته کنندگی آهن
FRAP	بر روی آنها صورت گرفتند. نتایج: تعیین پروفایل ترکیبات شیمیایی توسط کروماتوگرافی گازی متصل به طیف سنج
	جرمی نشان داد که اجزای اصلی تشکیل دهنده ی اسانس گیاه پاغازه اسپاتولنول و پالمیتیک اسید هستند. ترکیبات
	اصلی گیاه خوشاریزه گاما-دکالاکتون و ای-سزکویی لاواندولول می باشند. عصاره ی اندام های هوایی گیاه پاغازه در
	تمامی تست ها قدرت آنتی اکسیدانی بیشتری نسبت به سایرین از خود نشان داد و در رتبه ی بعد عصاره ی اندام های
	هوایی خوشاریزه قرار داشت. نتایج تست های DPPH، FRAP و بتاکاروتن-لینولئیک اسید به خوبی با یکدیگر
	همبستگی داشتند، اما با نتایج تست شلاته کنندگی آهن تاحدودی متفاوت بودند. نتیجه گیری: نتایج این تحقیق نشان
	داد که گیاهان پاغازه و خوشاریزه حاوی خواص آنتی اکسیدانی قابل توجهی هستند؛ لذا استفاده از آنها در صنایع
	غذایی و دارویی به عنوان عوامل نگهدارنده، مثرم ثمر خواهد بود.

مخفف ها: GC-MS، کروماتوگرافی جرمی متصل به طیف سنج جرمی؛ FRAP، آزمون قدرت کاهشی آنتی اکسیدانی فریک؛ DPPH، ۲،۲-دی فنیل-۱-پیکریل هیدرازیل

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تاریخ دریافت: ۲۲ اردیبهشت ۱۴۰۱؛ تاریخ دریافت اصلاحات: ۲۵ تیر ۱۴۰۱؛ تاریخ پذیرش: ۲۵ تیر ۱۴۰۱

doi: [10.52547/jmp.21.83.19](https://doi.org/10.52547/jmp.21.83.19)

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