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Original Article

Phytochemicals, Phenolic Profiles, Antioxidant and Antibacterial Activities of *Ferulago macrocarpa* Extracts from Lorestan

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Abstract

Ferulago macrocarpa (Fenzl) Boiss. commonly known as Chavil e Roshanball in Persian is a medicinal and aromatic plant from the Apiaceae family that grows in the west of Iran. The aim of this study was to investigate the phytochemical components, phenolic contents, antioxidant and antibacterial activities of hydroalcoholic extracts of *F. macrocarpa* flower and leaf from Lorestan, Iran.IC50 values, total phenol, flavonoid and anthocyanin contents of flower and leaf extracts were 481.75 and 1505.59 µg/mL, 59.33 and 45.57 mg Gallic acid equivalents, 45.76 and 11.53 mg Catechin equivalents and 0.73 mg Cyanidin-3-glucoside per g of dried extract, respectively. The flowers extract exhibited inhibitory effects on *Bacillus cereus* and *Staphylococcus aureus.* Analysis of the flower and leaf extracts by Gas chromatography-mass spectrometry (GC–MS) resulted in twenty-seven molecules consisting 97.42% of the total flowers extract volatile, and forty-four molecules consisting 94.91% of the total leaves extract volatile. The major components were bornyl acetate (37.1%), terpinolene (9.99%), thymol (7.46%) and limonene (6.39%) in the flower extract, and bornyl acetate (37.91%), o-cymene (7.83%), 2-hexanal (7.01%) and camphene (5.57%) in the leaf extract.

Keywords: *Ferulago macrocarpa*, Composition, Phenols, Antioxidant, Antibacterial

Introduction

Free radicals are atoms or molecules with unpaired electrons in their valence-shell and therefore are highly reactive particles. These particles are generated as by-products of intracellular biochemical reactions, as well as by many exogenous agents such as environmental pollution, ultraviolet radiation, cigarette smoking, alcohol consumption and inflammatory disorders [1]. Free radicals in low and moderate amounts play an important role in metabolic processes like phagocytosis, decreasing inflammation, cell division and transmission of signals. Nevertheless, their excessive accumulation causes changes in the structure and function of biological molecules including nucleic acids, proteins, lipids and carbohydrates, which can lead to various diseases like diabetes mellitus, cancer, cardiovascular disease and aging [2]. Antioxidants are compounds that by absorbing and eliminating free radicals may prevent or delay some types of cellular damage [3]. A balance between free radicals and antioxidants is essential for normal physiological function. In living organisms, enzymatic antioxidants including catalase, glutathione peroxidase, superoxide dismutase, and non-enzymatic antioxidants such as ascorbate, tocopherol, and glutathione are involved in removing, neutralizing or scavenging free radicals and protecting cells from their damaging effects [4]. However, exogenous antioxidants are also needed for the intact functioning of endogenous antioxidants. Due to the potential toxicity and carcinogenicity of synthetic antioxidants, there is an increasing tendency to replace them with natural harmless types of plant, animal and microbial sources [5]. Many plants contain high levels of polyphenols. Polyphenols are

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the compounds holding one or more phenol rings. These compounds can be involved in the neutralization of free radicals due to their reducing properties. The main classes of polyphenols are phenolic acids, flavonoids, stilbenes and lignans [6,7].

The genus *Ferulago* from Apiaceae family consists of about thirty-five species distributed in the world especially in the west of Iran, Turkey, and Iraq. About eight species are found in Iran, of which three are native to Iran [8]. *Ferulago* species have shown antibacterial, anti-cancer, antioxidant and anti-acetylcholinesterase properties [9,10,11,12]. In traditional Asian medicine, they have been used to treat wounds, snake bites, headaches, gastrointestinal tract and spleen diseases [13], also as sedative, tonic, aphrodisiac and digestive agents [14]. *Ferulago macrocarpa* (Fenzl) Boiss. (Chavil e Roshanball in Persian) is an aromatic plant growing in the west of Iran. It is a perennial glabrous herb with 40-100 cm tall, cylindrical dichotomously branched stem, shortly petiolate, pinnate-sect, terminal segment, linear-oblong, acute leaves, and yellowish, synflorescence corymbose-paniculiform flowers [15]. *F. Macrocarpa* has been used as a medicinal plant, food protectant and flavoring agent [13]. In the present study, volatile fraction composition, antioxidant and antibacterial properties, total phenol, flavonoids and anthocyanins of hydroalcoholic extracts of the *F. macrocarpa* flower and leaf from the Kouhdasht region of Lorestan province in the west of Iran were investigated for the first time.

Material and Methods

Plant Material and Extraction Procedure

The aerial parts of *F. Macrocarpa* were gathered from the Kouhdasht region (Lorestan province, west of Iran). Authentication of the plant and voucher specimen (No. 24011) was conducted by Tehran university herbarium, Iran. To prepare plant extracts, 10 g of each flower and leaf were extracted three times with ethanol/water (80:20) in the cycle of 24 hours each. The filtrates of extracts were combined and dried under a vacuum in a rotary evaporator at 40 ˚C. The obtained extracts (2.5 g of flower and 2.2 g of leaf) were stored at 4 ˚C until analyzed.

Antioxidant Activity

Antioxidant activity of the *F. Macrocarpa* extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free and stable radicals [16]. Briefly, 20 µL of the sample at various concentrations (250-1000 µg/mL) was mixed with 500 µL of the methanol solution of DPPH (6×10^{-6} M) and 480 µL of methanol. These solutions were vortexed thoroughly and incubated in dark for 30 minutes at room temperature. Antioxidant activity was determined by measuring the absorbance at 517 nm using UV-Vis spectrophotometer (JENWAY 6405, UK) against methanol as blank. The radical *scavenging* percentage was calculated using the following formula:

$$\%\,\mathrm{RSA} = \frac{AC - AS}{AC} \times 100 \quad (1)$$

Where Ac is the absorbance of DPPH as control and As is the absorbance of DPPH in the presence of the test sample. The needed concentration of sample for neutralizing 50% of free radicals (IC50) was obtained using the DPPH radical inhibitory curve against the sample concentration.

Total Phenolic Content (TPC)

The TPC of the *F. Macrocarpa* extracts was assessed by the Folin-Ciocalteu colorimetric method, based on the reaction of Folin reagent with hydroxyl active groups in phenolic compounds [17]. Briefly, 0.5 mL of the extract or Gallic acid standard solution at different concentrations was mixed with 2 mL of 1:10 diluted Folin-Ciocalteu reagent. The mixture was then neutralized with 4 mL of sodium carbonate solution (7.5%). After 30 minutes of incubation in the dark with intermittent shaking, the absorbance was measured at 765 nm against the blank using UV-Vis spectrophotometer. The total phenolic content was calculated from the calibration curve of Gallic acid (**S1**) (Y=0.0077X +0.0051 (R^2=0.9995) and expressed as mg of Gallic acid equivalents (GAE) per g of dry extract.

Total Flavonoid Content (TFC)

The TFC of the *F. Macrocarpa* extracts was measured by the aluminum chloride colorimetric assay [18]. 1 mL of the extract or Catechin standard solution at different concentrations was dispensed in a flask containing 4 mL of distilled water. To the above mixture, 300 µL of NaNO2 (5%) was added. After 5 minutes, 300 µL of AlCl3 (10%) was added, allowed to stand for 6 minutes, then followed by the addition of 2 mL of NaOH (40%). The final volume of the solution was adjusted to 10 mL with distilled water and thoroughly mixed.

After 15 minutes incubation at room temperature, the absorbance was recorded against the blank at 510 nm using UV-Vis spectrophotometer. The total flavonoid content was calculated from the standard curve of Catechin(S2) ($Y = 0.0026 X + 0.005$ ($R^2 = 0.9927$)) and expressed as mg of Catechin equivalents (CAT) per g of dry extract.

Total Monomeric Anthocyanin content (TMA)

The TMA content of the *F. Macrocarpa* extracts was estimated by the pH differential method [19]. This method is based on the fact that anthocyanins absorb light at 510 nm at pH 1.0 but not at pH 4.5. Thus, the difference in absorbance at 510 nm is directly proportional to anthocyanin concentration [20]. The extracts were diluted in a 1:12 ratio (*v/v*) with potassium chloride and sodium acetate buffers (pH 1.0 and 4.5, respectively) in separate vessels. After 15 minutes incubation at room temperature, the absorbance of each solution was read spectrophotometrically at 510 nm. The concentration of anthocyanins was calculated using the following equation:

Anthocyanin (mg/L) = $(A \times MW \times DF \times 10^3) / (\varepsilon \times L)$

Where $A=A_{pH1}- A_{pH4.5}$; MW=484.82 g/mol for Cyanidin-3-glucoside (C3G); DF is dilution factor; Molecular absorption coefficient (ε)= 24825 L mol^{-1} cm^{-1} for C3G; L= path length in cm; and 10^3 = factor for conversion from g to mg.

Total phenol, flavonoids, and anthocyanins of the *F. Macrocarpa* extracts were measured at concentrations of 250-1000 µg/mL, at 5 °C, 25 °C and 45 °C, at zero and after 24 hours incubation at room temperature.

Antibacterial Activity

Antibacterial activity of the *F. Macrocarpa* extracts was measured against *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumonia* (ATCC 10031) and *Escherichia coli* (ATCC 8739) by disc diffusion method [21]. Nutrient agar plates were inoculated with the bacterial strains using a sterile swab. 30 µL of the extracts (1-100 mg/mL) was placed on the surface of the inoculated plates. Amikacin and DMSO were used as positive and negative control, respectively. After 24 hours incubation at 37 °C, the diameter of the inhibition zones was measured (mm).

All determinations were carried out in triplicate and the results were presented as mean.

Chemical Characterization

The volatile fraction composition of the *F. Macrocarpa* flower and leaf extracts were analyzed using a gas chromatograph equipped with a GCMS-QP5050A mass spectrometer (Shimadzu GC-17A, Kyoto, Japan). Separation of the compounds was carried out on a BP-X5 fused-silica capillarity column with the length of 30 m, the inner diameter of 0.22 mm and the film thickness of 0.25 µm. Ultra-pure helium at a flow rate of 0.8 mL/min was used as the carrier gas. 1 µL of the extract (10 mg/mL) was injected using a 50:1 split ratio. Mass spectra were recorded at 70 eV over a scan range of 35-450 m/z. Injector and interface temperatures were 280 ºC and 260 ºC, respectively. The initial oven temperature was 40 ºC, gradually increased to 100 ºC at the speed of 20 ºC/min, and then raised to 220 ºC at 3 ºC/min and finally increased to 280 ºC at 30 ºC/min and held for 2 minutes.

The extract constituents were identified by comparison of their retention indices relative to (C8-C20) n-alkanes and by comparison of their mass spectra with those of the internal reference mass spectra library (NIST.08 and Wiley 9.0). The percentage of volatile compositions was calculated from the GC peak areas.

Results and Discussion

The antioxidant activity of the samples was tested using DPPH method. DPPH is a stable free radical and its methanolic solution shows a maximum absorption at 517 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, the color of the solution changes from dark purple to light yellow and hence the absorption of the solution decreases [22]. Flower extract with IC50 value of 481.6 µg/mL exhibited considerable activity against DPPH radicals. This activity was increased by increasing the extract concentration. Leaf extract with the IC50 of 1505.6 µg/mL showed less antiradical activity than the flower extract (Table 1). Since the antioxidant property of the tested samples may be due to their content of phenolic compounds, the level of these compounds in the extracts was measured in the current study. The effects of extract concentration, ambient temperature and incubation time on their amount were also investigated. Phenolic acids and flavonoids generally act as antioxidants by trapping free radicals. Anthocyanins, a class of flavonoids,

are potent antioxidants and free radical scavengers [23].

The results of this study showed the *F. macrocarpa* flower and leaf hydroalcoholic extracts contain a considerable level of phenolic compounds (Table 1). With increasing concentration of the extracts, the amount of extracted phenols and flavonoids increased. However, the level of extracted anthocyanins decreased in flower extract. By diluting the flower extract and increasing the ratio of solvent to dry extract, the extraction level of anthocyanins increased. A higher solvent ratio may result in faster anthocyanin dissociation. In another study, the amount of anthocyanin extraction increased with increasing solvent content [24]. The extracted phenol level in the flower and leaf extracts increased with temperature rise up to 25 °C but decreased at higher temperatures. Mild heat may cause softening of plant tissues and weaken the interaction of phenolic compounds with other compounds and thus facilitate the extraction process of compounds [25]. However, higher temperatures may degrade heat-sensitive polyphenols [26]. The extracted flavonoid and anthocyanin levels showed a reverse correlation with temperature. At high temperatures, certain flavonoids may decompose into other compounds. Najar and colleagues observed that in regions with lower temperatures, the accumulation of flavonoids in the plant is greater [27]. Monomeric anthocyanins may be polymerized by the increase in temperature, and because polymers do not exhibit color change with changing pH, they cannot be measured by the differential pH method [19].

The results showed that increasing incubation time reduced the amount of phenol, flavonoids, and anthocyanins of the extracts, which probably resulted from the chemical decomposition of these compounds in the prolongation of extraction time.

Given that *F. macrocarpa* is used as a food preservative, the effect of its extracts on food pathogens including two Gram-positive bacteria (*B. cereus and S. aureus*) and two Gram-negative bacteria (*K. pneumonia* and *E. coli*) were studied. In the present experiment, the flower hydroalcoholic extract of *F. Macrocarpa* exhibited antibacterial activity against *S. aureus* with mean inhibition zone diameter 11 and 13 mm, and *B. cereus* with mean inhibition zone diameter 10 and 11 mm at concentrations of 1.5 and 3 mg/disc, respectively. The *F. macrocarpa* flower and leaf extracts did not show any activity against Gram-negative bacteria (Fig.1). This difference may be due to the presence of an external membrane in addition to the peptidoglycan layer in the wall of the Gram-negative bacteria. The hydrophilic surface of this membrane acts as a barrier to antibiotic penetration. The cell wall of the Gram-positive bacteria lacks an outer membrane and therefore it is easier to cross the cell wall [28].

The antimicrobial effects of some species of the genus *Ferulago* have already been reported.

Tabataba'I Yazdi has reported that ethanolic and aqueous extracts of *F. angulata* have completely stopped the growth of *S. epidermium* and *Y. enterocolitica*, but did not significantly affect the growth of *E. aerogenes* [29]. Research carried out by Chalabian *et al*. showed that *F. bernardii* extract was inhibitory against *S. aureus*, *B. subtilis*, *E. coli*, and *C. albicans*, but was not active against *S. epidermidis* and *S. saprophyticus* [30]. Flower extract analysis of the *F. macrocarpa* showed twenty-seven constituents, representing 97.42% of the total volatile fraction.

Table 1 Radical scavenging activity, Phenol, Flavonoid and Anthocyanin contents of the *Ferulago macrocarpa* (Fenzl) Boiss. flower and leaf hydroalcoholic extracts.

Extract	Con. (µg/ml)	RSA (%)	Phenols (mg.GAE/g dw)			Flavonoids (mg.CAT/g dw)			Anthocyanins (mg.C3G/g dw)		
			5 °C	25 °C	45 °C	5 °C	25 °C	45 °C	5 °C	25 °C	45 °C
Flower	250	32	8.1	22.6	12.2	36.2	21.5	21.2	5.12	2.19	1.46
	500	51	20.5	29.2	28.9	60	28.5	27.3	4.15	1.7	0.97
	1000	94	46.9	59.3	48.1	116.5	45.8	41.5	1.7	0.73	0.48
Leaf	250	39	12.5	15.3	10.9	8.1	5.5	3.8	2.19	1.9	0.48
	500	41	23.3	24.1	19.9	12.3	8.5	7.7	2.44	2.19	0.73
	1000	45	41.2	45.6	40.3	18.5	11.5	8.5	2.92	2.68	1.22

RSA represents DPPH radical scavenging activity, GAE mg/g dw, CAT mg/g dw, and C3G mg/g dw represent mg of Gallic acid equivalents, mg of Catechin equivalents and mg of Cyanidin-3-glucoside equivalents per g of dried extract,respectively.

Table 2 Composition of the *Ferulago macrocarpa* (Fenzl) Boiss. flower hydroalcoholic extract.

No.	Component[a]	RT[b]	RI[c]	Area%
1	alpha Pinene	4.81	939	5.37
2	Camphene	5.17	953	2.31
3	Sabinene	5.55	976	1.94
4	Myrcene	5.75	991	5.76
5	Mesitylene	6.04	994	0.29
6	alpha Phellandrene	6.22	1005	5.99
7	o-Cymene	6.63	1022	5.26
8	Limonene	6.70	1031	6.39
9	beta Phellandrene	6.79	1031	2.43
10	z beta Ocimene	6.94	1040	0.45
11	gamma Terpinene	7.33	1062	0.47
12	Terpinolene	8.01	1088	9.99
13	2,5-Dimethylstyrene	8.23	1096	0.23
14	Camphor	10.01	1143	0.13
15	Borneol	10.70	1165	2.04
16	p-Cymene-8-ol	11.09	1183	0.73
17	Pulegone	12.82	1237	0.25
18	Bornyl acetate	14.22	1285	37.08
19	Thymol	14.49	1290	7.46
20	Carvacrol	14.83	1298	0.58
21	Copaene	17.57	1376	0.07
22	beta Elemene	18.03	1391	0.19
23	Aromadendrene	19.15	1439	0.38
24	E Caryophyllene	19.39	1417	0.49
25	z beta Bisabolene	21.92	1504	0.39
26	Spathulenol	25.76	1576	0.25
27	Caryophyllene oxide	25.96	1581	0.57

[a]Compounds identified in the BP-X5 capillary column; [b]Retention time in minutes; [c]Literature retention indices.

Table 3 Composition of the *Ferulago macrocarpa* (Fenzl) Boiss. leaf hydroalcoholic extract.

No.	Compound[a]	RT[b]	RI[c]	Area (%)
1	2-Hexenal	3.58	857	7.05
2	Hexanol	3.77	867	2.56
3	Nonane	4.11	899	0.22
4	Heptanal	4.31	899	0.22
5	α- Thujene	4.65	931	0.51
6	α- Pinene	4.83	939	3.68
7	Camphene	5.17	953	5.57
8	Sabinene	5.54	976	2.06
9	Myrcene	5.73	991	2.92
10	3-Hexanol acetate	6.06	1004	0.12
11	α- Phellandrene	6.21	1005	1.13
12	α-Terpinene	6.42	1018	0.17
13	ortho Cymene	6.62	1022	7.83
14	Limonene	6.69	1031	1.64
15	β - Phellandrene	6.78	1031	0.58
16	(z) β - Ocimene	6.93	1040	0.94
17	γ -Terpinene	7.32	1062	0.59
18	Terpinolene	7.99	1088	1.92
19	Nonanal	8.41	1102	0.17
20	Limonene oxide	8.83	1139	0.13
21	Verbenol	9.78	1140	0.65
22	Camphor	9.98	1143	0.25
23	Borneol	10.68	1165	2.42
24	P-Cymene-8-ol	11.07	1183	0.77

25	Decanal	11.36	1204	0.30
26	Myrtenal	11.47	1193	0.11
27	Verbenone	11.88	1204	0.26
28	Carveol	12.08	1229	0.13
29	Pulegone	12.81	1237	0.52
30	Decanal	13.58	1272	0.37
31	Bornyl acetate	14.28	1285	37.91
32	Thymol	14.50	1290	1.66
33	Undecanol	17.30	1372	0.45
34	Copaene	17.56	1376	0.84
35	β - Elemene	18.00	1391	1.30
36	Undecanal	18.73	1407	0.79
37	Aromandendrene	19.38	1439	0.82
38	Geranyl acetone	20.23	1453	0.24
39	Caryophyllene	20.82	1417	0.12
40	Dodecanol	21.25	1473	0.16
41	Germacrene D	21.80	1480	0.97
42	β - Selinene	22.18	1485	0.15
43	α- Bisabolene	22.39	1504	0.15
44	α- Farnesene	22.59	1508	0.20
45	δ - Cadinene	23.16	1524	0.22
46	Nerolidol	24.66	1564	0.23
47	Spathulenol	25.78	1576	1.00
48	Carophyllene oxide	26.00	1581	1.91

[a]Compounds identified in the BP-X5 capillary column; [b]Retention time in minutes; [c]Literature retention indices.

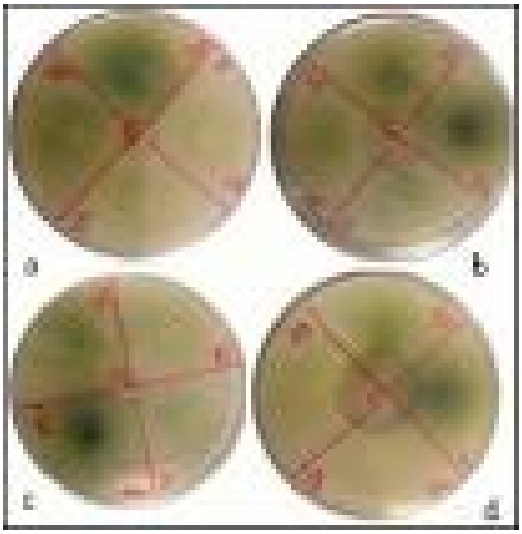

Fig. 1 Antibacterial activity of the *Ferulago macrocarpa* (Fenzl) Boiss. flower extract against (a) *K. pneumonia*, (b) *S. aureus*, (c) *E. coli*, (d) *B. cereus.*

The main classes present in the flower extract were monoterpene hydrocarbons (46.62%) and oxygenated monoterpenes (48.31%) in a total of nineteen compounds. Five sesquiterpene hydrocarbons (1.53%), two oxygenated sesquiterpenes (0.83%) and one non-terpene compound were also identified. The flower extract comprised mainly of bornyl acetate (37.08%), followed by terpinolene (9.99%), thymol (7.46%), limonene (6.39%), α-phellandrene (5.99%), myrcene (5.76%), α-pinene (5.37%) and *o*-cymene (5.26%) as shown in Table 2.

Antibacterial activity of the flower extract may be attributed to the presence of terpinolene, thymol and α- pinene. The weak antibacterial activity of these compounds has been reported [31].

The identified components in the volatile fraction of *F. Macrocarpa* leaves extract are presented in Table 3. The volatile fraction of leaves extract comprised forty-eight constituents accounting for 94.91% of the total volatile amount. Of the detected compositions, 74.59% was monoterpenes and 7.91 % was sesquiterpenes, representing a total of 82.50%. The main identified compounds were bornyl acetate (37.91%), o-cymene (7.83%), 2-hexanal (7.01%), camphene (5.57%) and α-pinene (3.64%). The results indicated that bornyl acetate was the major component in the volatile obtained from both flower and leaf extracts of *F. macrocarpa*. There are no investigations on volatile of the *F. macrocarpa* flower and leaf extract from Lorestan (Iran), while the volatile composition of aerial parts of *F. macrocarpa* growing in Ilam (western Iran) have previously been studied. Sajjadi *et al*. (2012) reported the major component of *F. macrocarpa* fruit essential oil was bornyl acetate (40.8%), followed by 2,3,6-trimethyl benzaldehyde (7.2%) and δ-selinene (5.5%). Monoterpenes and

sesquiterpenes comprised 63.6% and 27.5% of the *F. macrocarpa* fruit essential oil, respectively [8]. Results of another study by Hadjiakhoondi *et al*. (2002) indicated that bornyl acetate (45.7%), borneol (17.2%) and β-gurjunene (9.2%) are the main compounds of the *F. macrocarpa* aerial parts essential oil [32]. Jila Asghari *et al*. (2013) detected borneol and bornyl acetate in the *F. macrocarpa* aerial parts *essential oil [33]*.

Conclusion

F. macrocarpa is an aromatic herb and a rich source of phenolic and terpenoid compounds with antioxidant and antibacterial capacities. Thus, it may be used in cosmetics, food and pharmaceutical industries.

Conflict of interest

There are no conflicts of interest regarding this manuscript.

Acknowledgments

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References

1. Sosa V, Moliné T, Somoza R, Paciucci R, Kondoh H, Leonart M E. Oxidative stress and cancer: an overview. Ageing Res. Rev. 2013;12:376-390.
2. Khalili M, Ebrahimzadeh MA. A review on antioxidants and some of their common evaluation methods. JMUMS. 2015;24:188-208.
3. Sharififar F, Moshafi MH, Mansouri SH, Khodashenas M, Khoshnoodi M. In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multi flora* Boiss. Food control. 2007;18:800-805.
4. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 2003;91:179-194.
5. Alavi L, Barzegar M, Jabbari A, Naghdi Badi H. Effect of heat treatment on chemical composition and antioxidant property of *Thymus daenensis* essential oil. JMPR. 2010;3:129-138.
6. Tuyen PT, Xuan TD, Khang DT, Ahmad A, Quan NV, Tu Anh TT, Minh TN. Phenolic Compositions and Antioxidant Properties in Bark, Flower, Inner Skin, Kernel and Leaf Extracts of *Castanea crenata Sieb*. et *Zucc*. Antioxidants. 2017;6:31-44.
7. Karaman Ş, Tütem E, Başkan K S, Apak R. Comparison of total antioxidant capacity and phenolic composition of some apple juices with combined HPLC-CUPRAC assay. Food Chem. 2010;120:1201-1209.
8. Sajjadi S, Shokoohinia Y, Jamali M. Chemical composition of essential oil of *Ferulago macrocarpa* (Fenzl) Boiss. fruits. RPS. 2012;7:197-200.
9. Basile A, Sorbo S, Spadaro V, Bruno M, Maggio A, Faraone N, Rosselli S. Antimicrobial and antioxidant activities of coumarins from the roots of *Ferulago campestris* (Apiaceae). Molecules. 2009;14:939-952.
10. Amirghofran Z, Bahmani M, Azadmehr A, Javidnia K. Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. Neoplasma. 2006;53:428-433.
11. Abedini A, Roumy V, Mahieux S, Gohari A, Farimani M M, Rivière C, Hennebelle T. Antimicrobial activity of selected Iranian medicinal plants against a broad spectrum of pathogenic and drug multi resistant micro-organisms. J. Appl. Microbiol. 2014;59:412-421.
12. Dall'Acqua S, Maggi F, Minesso P, Salvagno M, Papa F, Vittori S, Innocenti G. Identification of non-alkaloid acetylcholinesterase inhibitors from *Ferulago campestris* (Besser) Grecescu (Apiaceae). Fitoterapia, 2010;81:1208-1212.
13. Sajjadi SE, Jamali M, Shokoohinia Y, Abdi G, Shahbazi B, Fattahi A. Antiproliferative evaluation of terpenoids and terpenoid coumarins from *Ferulago macrocarpa* (Fenzl) Boiss. fruits. Phcog Res. 2015;7:322-328.
14. Rafieian-kopaei M, Shahinfard N, Rouhi-Boroujeni H, Gharipour M, Darvishzadeh-Boroujeni P. Effects of *Ferulago angulata* extract on serum lipids and lipid peroxidation. Evidence-Based Complementary & Alter Med. 2014;680856:1-4.
15. Taran M, Ghasempour HR, Shirinpour E. Antimicrobial activity of essential oils of *Ferulago angulata* subsp. *carduchorum*. Jundishapur J Microbiol. 2010;3:10-14.
16. Jiménez-Escrig A, Jiménez-Jiménez I, Sánchez-Moreno C, Saura-Calixto F. Evaluation of free radical scavenging of dietary carotenoids by the stable radical 2, 2-diphenyl-1-picrylhydrazyl. J Sci Food Agric. 2000; 80:1686-1690.
17. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc. 2007;2:875.
18. Kumar S, Kumar D, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. Acta Pharm. 2008;58:215-220.
19. Giusti MM, Wrolstad RE. Characterization and measurement of anthocyanins by UV-visible spectroscopy. CPFAC. Wiley, 2001.
20. Wrolstad R E, Durst R W, Lee J. Tracking color and pigment changes in anthocyanin products. Trends food sci tech. 2005;16:423-428.

21. Li Z, Lee D, Sheng X, Cohen RE, Rubner MF. Two-level antibacterial coating with both release-killing and contact-killing capabilities. Langmuir. 2006;22:9820-9823.
22. Yuan JP, Li X, Xu SP, Wang JH, Liu X. Hydrolysis kinetics of secoisolariciresinol diglucoside oligomers from flaxseed. J Agric Food Chem. 2008;56:10041-10047.
23. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. Rsc Advances. 2015;5:27986-28006.
24. Cacace J E, Mazza G. Mass transfer process during extraction of phenolic compounds from milled berries. J Food Eng. 2003;59:379-389.
25. Shi J, Yu J, Pohorly J, Young JC, Bryan M, Wu Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. J Food Agric Environ. 2003;1:42.
26. Wissam Z, Ghada B, Wassim A, Warid K. Effective extraction of polyphenols and proanthocyanidins from pomegranate's peel. Int J Pharm Pharm Sci. 2012;4:675-682.
27. Najar M, Hemmati KH, Khorasaninejad S, Bagherifard A. The effect of altitude on the morphological and biochemical characteristics of *Urticadioica* L. in Mazandaran and Golestan province. Iranian Journal of Plant Ecophysiology. 2014;9:1-11.
28. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. Int J Antimicrob Agents. 2001;17:527-529.
29. Tabatabaei Yazdi F, Alizade Behbahani B, Heidari Sureshjani M. The comparison of antimicrobial effects of Chevil (*Ferulago angulata*) extract with a variety of common therapeutic antibiotics in vitro. J Arak Uni Med Sci. 2014;17:35-46.
30. Chalabian F, Monfared A, Larijani K, Saldozi S. Comparison of the matter in the three plants *Ferulago subvelutin*, *Chenopodiumbotrys* L., *Rechandrosagallica*, investigate their antimicrobial activity against some pathogenic bacteria. J Med Aromatic Plant Res Iran. 2005;2:146-154.
31. Sokmen A, Gulluce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, Sahin F. The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food control. 2004;15:627-634.
32. HAJI AA, Aghel N, Etemadi R. Chemical and biological study of essential oil of *Ferulago macrocarpa* (Fenzi) Boiss. Hamdard Med. 2002;45:35-38.
33. Asghari J, Salehi M, Mazaheri Tehrani M. Isolation of borneol and bornyl acetate from Ferulago macrocarpa by microwave irradiation. JHD. 2013;4:89-94.