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Original Article

Variation of Eryngo (*Eryngium caeruleum* M.Bieb.) Essential Oil Content and Biological Activity in Wild and Cultivated Conditions

Foroogh Mirzania[1], Javad Ghasemian Yadegari[2*], Iraj Salimikia[2*], Yaghoub Sarrafi[3], Atousa Aliahmadi[4] and Javid Aalaei[5]

[1]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Lorestan Province, Iran*
[3]*Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran*
[4]*Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran*
[5]*Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran*

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***Corresponding author**
Jvd.ghasemian@gmail.com,
Irajsalimikia@yahoo.com

ABSTRACT

In this investigation essential oils of the wild and cultivated *Eryngium caeruleum* M.Bieb. were analysed by GC and GC/MS, and screened for their antimicrobial activities. At least seventy-three ingredients were identified in both specimens that limonene (16.4-32.3%), β-bisabolol (10.3-17.3%), δ-3-carene (6.6-9.4%), cis-limonene oxide (3.1-7.8%), (E)-β-farnesene (4.4-4.8%) and β-sesquiphellandrene (2.4-2.9%) were the major components. Also, the antibacterial and the antimycotic activities of these oils were reported against six bacterial and fungal strains. The antibacterial analysis displayed that both oils presented high activity versus all the tested Gram-negative strains in a range of MIC values from 4 to 8 mg/ml. The antifungal test results proved moderate activity against *Candida krusei* and *Candida albicans* (MIC values 1 to 4 mg/ml). Our results, have been proved that cultivation and domestication of this plant seems improved the microbial test results for the cultivated sample. These are aspects that turn this plant into useful crops for domestication and commercialization. Results of this investigation propose that it is noteworthy to grow this herb by the farmers in Babol, Mazandaran, Iran for pharmaceutical, therapeutic and food targets and natural bactericide agent.

INTRODUCTION

The Apiaceae is one of the greatest plant families in the world. This family contains nearly 450 genera and 3700 species throughout the world. Members of this family usually contain a specific spicy or aromatic odor which is owing to the presence of essential oil [1-4]. Plants of Apiaceae encompass diverse compounds with many biological properties. Some of the principal specifications are capable to induce apoptosis, cyclooxygenase inhibitory, vaso-relaxant, hepatoprotective, antibacterial, and antitumor activities [5-7]. Iran with exclusive climatic conditions has a large diversity of plants, in particular a vast variety of Apiaceae species. This family has 121 genus and 360 species in Iran. *Eryngium* is the biggest and undoubtedly the most taxonomically intricate genus of this family. *Eryngium* genus consists of nearly 250 species that widespread in Eurasia, North Africa, North and South America, and Australia [8]. Some species of this large genus are scarce or critically endangered for example, *E. alpinum* L., *E. aristulatum*, *E. constancei* Sheikh, *E. cuneifolium* and *E. viviparum* [9, 10].

The species of this genus are cultivated as decorative species, vegetable and pharmaceutical products for folk applications. *Eryngium* species are applied as spices and are cultivated all over the world and have been used in traditional medicines in some countries for procurement of laxative, appetite stimulant, diuretic, antinociceptive or anti-inflammatory drugs and for the cure of malaria, hypertension, asthma, gastrointestinal problems, fevers, burns, diarrhea, etc [11]. In recent years, with further chemical and biological researches, *Eryngium* genus has revealed its potential as

medicinal crops. Eryngo (*E. caeruleum*) is a vegetative and perennial herb scattered in the northern provinces of Iran [12]. The plant aerial parts are usually employed in medicine and food crafts in Iran [13]. Young and pristine leaves are applied as a vegetable and also as stuffing in the preparation of various local foods [14]. *E. caeruleum* has several pharmaceutical characteristics including tonic, paregoric, sedative, anti-flatulence, diuretic and appetizer [15, 16]

During recent years, wild populations of this medicinal plant have declined so drastically that it is now considered threatened. This situation is attributed essentially to over-harvesting. A good solution to prevent the extinction of this medicinal plant is domestication. Nevertheless, the domestication of wild medicinal species needs a comprehensive understanding of the effect of cultivation on their chemical constituents and therefore on their biological properties. Changes in the growth condition of the plant, like those associated with transplanting from a wild ecosystem to a cultivated field, can result in changes in herb growth and chemical composition. In this research the essential oils of the wild and cultivated *E. caeruleum* were extracted and compared to examine the cultivation influence on chemical composition and biological properties of essential oils of this medicinal species.

MATERIALS AND METHODS

Plant Materials

Aerial parts of *E. caeruleum* were accumulated (1300 g) in full flowering period from natural locations of Babol, Mazandaran, Iran (Latitude: 36°33′04″ N, Longitude: 52°40′44″ E and Elevation above sea level: -1 m). A voucher sample (MPH-2368) is registered in the herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. Harvested portions were dehumidified at the ambient temperature in shade for two weeks, and grounded into a powder before extraction of essential oil.

Extraction of Essential Oil

Air-dried and crushed herb substance (200 g) of each sample was subjected to hydrodistillation for 3.5 h using a Clevenger-type system pursuant to the way presented in pharmacopoeia (British Pharmacopoeia, 1988). The essential oils obtained were separated and dried over anhydrous sodium sulfate, then stored at 4 °C in the dark until use.

Gas Chromatography-Mass Spectrometry

The samples were analyzed by GC/FID using a Thermoquest-Finnigan instrument with a Flame Ionization Detector (FID). The analyses were performed in DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm) capillary column. The flow rate of the carrier gas (Nitrogen) was 1.1 ml/min. The temperature of oven was regulated at 60 ºC for 1 min, then organized to 250 ºC at a velocity of 4 ºC /min, and afterwards fixed for 10 minutes. A split injection with a ratio 1:20 was used with the injector temperature fixed at 250 ºC and the detector temperature at 280 ºC.

GC-MS analyses were performed with Thermoquest-Finnigan Trace GC-MS device with helium as the carrier gas at a constant linear velocity of 1.1 ml/min. The quadrupole mass spectrometer was scanned above 45-465 amu with an ionization voltage of 70 eV and an ionizating flow of 150 μA. The transfer line temperature was 250 ºC. The column used was DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was increased from 60 ºC to 250 ºC with rapidity of 5 ºC/min, and then fixed at 250 ºC for 10 min.

The components were identified by comparison of their mass spectra with authentic reference compounds where possible and by reference to Wiley and Adams library, as well as by comparison of their retention indices (RI) with literature data [14].

Antibacterial and Antifungal Activity

In vitro antibacterial activities of essential oils was calculated versus *Enterococcus faecium* clinical strain and *Staphylococcus aureus* ATCC (American Type Culture Collection) 25923 as Gram positive bacteria and *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC58327 as Gram negative bacteria. Determination of MIC (Minimum Inhibitory Concentration) quantity was done by broth micro-dilution manner as reported by CLSI (Clinical Laboratory and Standard Institute) with some changes [17-19]. In general, a serial dilution of each oil was generated in a concentration range of 64 to 0.125 μL/ml in sterile 96 wells trays including Mueller-Hinton broth medium completed by 0.5% Tween 80 as co-solvent. Normal saline was applied for generation of inoculants having turbidity

equivalent to 0.5 McFarland. The inoculants of the bacterial strains were produced from newly cultured bacteria that were adjusted to 0.5 McFarland turbidity and were more diluted (1:100) applying MHB (Mueller-Hinton Broth) medium merely before increasing to the serial diluted specimens. Trays incubated for 24 h at 37 °C. MIC quantities were registered as the lowest concentrations which could inhibit clearly visible growth of microorganisms [18].

Minimum bactericidal concentrations (MBCs) were determined by culturing of 100 µL of each no-growth well on to nutrient agar plates and incubation at appropriate temperature. MBC values were registered as the lowest concentration which killing of 99.9 % of microorganism. Each experiment was accomplished in triplicate and Cefixime was applied as standard antibacterial reagent.

Two fungi: *Candida krusei* ATCC 5295 and *Candida albicans* ATCC 10231 were examined in this part of investigation. Evaluation of MIC was done by broth micro-dilution way as published by CLSI with some modifications (Ana, *et al*., 2007). In general, a serial dilution of each essential oil was produced in a concentration series of 64 to 0.125 µL /ml in sterile 96 wells plate including RPMI pH 7 supplemented by 2% (w/v) Dextrose, MOPS (3-(N-Morpholino) propanesulfonic acid) (0.165 M) and 0.5% Tween 80 as co-solvent for essential oil. Trays were incubated at 35 °C for 7 days. Afterwards, a 1:50 diluted stock of conidia was utilized for inoculation of plates including diluted essential oils. Plates incubated for 48 h at 30 °C. MIC quantities were registered as the lowest concentrations which could inhibit evident growth of microorganisms (Jorgensen, 2007). Minimum fungicidal concentrations (MFCs) were specified by culturing of 100 µL of each no-growth well onto Sabouraud dextrose agar plates and incubation at suitable temperature. MFC quantities were registered as the lowest concentration which concluded in killing of 99.9 % of experimented microorganism. Each experiment was done in triplicate and Nystatin applied as standard antifungal reagent.

RESULTS AND DISCUSSION

The essential oils were isolated by the hydrodistillation method in the yields of 0.5% w/w and 0.4% w/w for the wild and cultivated samples, respectively. The oil samples were analysed by GC and GC-MS. In total, seventeen and nineteen ingredients representing 78.8 and 81.3% of the oils were identified and quantified in the wild and cultivated sample, respectively. Peak recognition and relative values of the components are presented in Table 1. Almost, twenty-seven components were found at relative concentrations upper than 1% in both samples. The principal components found were limonene (16.4 and 32.3% in the oils from cultivated and wild herbs, respectively); β-bisabolol (17.3% and 10.3%), δ-3-carene (9.5% and 6.6%), cis-limonene oxide (7.8% and 3.1%), (E)-β-farnesene (4.4% and 4.8%), acorenone (3.5% and tr), phytol (2.9% and 1.8%) and β-sesquiphellandrene (2.4% and 2.9%). Monoterpenes were the dominant ingredients in the *E. caeruleum* oil from wild grown (49.1%) and cultivated plants (39.3%). Limonene and *δ*-3-carene were the principal monoterpenes in both oils followed by carvone (2.8% and 1.0%), trans-p-mentha-2,8-dien-1-ol (1.9% and 0.5%), cis-p-mentha-2,8-dien-1-ol (1.6% and 1.1%), β-pinene (0.2% and 1.8%) and trans-carveol (0.9% and 1.2%) in the essential oils from cultivated and wild plants, respectively. Sesquiterpenes ingredients represented the second chief class of components in the oil from cultivated *E. caeruleum* plant (28.1%) while in the wild sample was much less (21.5%). The quantities of some chief components of *E. caeruleum* oil were decreased in wild sample. Some principal compounds like cis-limonene oxide, β-bisabolol and δ-3-carene were increased in cultivated sample. The results showed some quantitative and qualitative differences between the two samples but components like limonene, β-bisabolol, δ-3-carene, cis-limonene oxide, (E)-β-farnesene, and β-sesquiphellandrene were the main compounds in both samples. Also essential oil outputs were very similar in both situations (1.7% and 1.5% for wild and cultivated samples, respectively), albeit herb growth was greater in the cultivated sample. It was inferred that the cultivated components profiles were approximately equivalent to wild plants.

Formerly, few researches presented the chemical components of the essential oils from *E. caeruleum* belonging to some areas in Iran. Hashemabadi *et al*. (2010) reported the essential oils compositions of this plant wild growing in Langeroud city, Guilan

province, in the north of Iran at different growth phases. The β-sesquiphellandrene (44.2%), limonene (18.4%) and β-bisabolene (6.1%) were main ingredients, respectively, at vegetative stage (May 2009) in the leafage of littoral herbs while 5-methyl-2-pyrimidone (53.8%) and β-sesquiphellandrene (11.3%) were principal compounds in hill slope herbs. The chief compounds of the essential oils in leafage of coastal herbs at vegetative stage (June 2009) were β-sesquiphellandrene (27.3%), limonene (14.3%) and 5-methyl-2-pyrimidone (14.1%). On the hill slope, herb ingredients were 4(5)-acetyl-1H-imidazole (50.1%), β-sesquiphellandrene (15.5%) and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (11.0%) [20]. Also, Hashemabadi and Kaviani (2011) in other research reported the main ingredients of the essential oils of leaves and stems of *E. caeruleum* plant in Lahidjan city, east of Guilan province, in the north of Iran, from coastal and hill slope regions at vegetative and generative stages. Results displayed that Fifty-five ingredients were identified in *E. caeruleum* oils.

The principal compounds were 3-hexyne (46.1%), β-sesquiphellandrene (20.4%) and limonene (10.7%) at first vegetative stage (May, 2009) in the leaves of coastal samples, and 5-methyl-2-pyrimidone (53.4%), limonene (12.8%) and 6-acetoxy-2, 3-dihydro-1H-pyrrolizin (12.4%) were major ingredients in hill slope sample. The main compositions at second vegetative stage (June, 2009) in the leaves of coastal samples were 4 (5)-acetyl-1H-imidazole (63.6%), thymol (13.9%) and β-sesquiphellandrene (10.0%), and in hill slope samples were β-sesquiphellandrene (44.3%), limonene (20.1%) and trans-β-farnesene (14.1%). 5-Methyl-2-pyrimidone (74.9%) and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (15.8%) were principal ingredients at generative stage (July, 2009) in the stems of coastal samples while β-sesquiphellandrene (25.8%), 5-methyl-2-pyrimidone (18.7%) and limonene (11.8%) were main ingredients in hill slope specimens [21]. With a quick look we would find that quantity and quality of ingredients were different than our report. Other species of *Eryngium* genus have been phytochemically examined like *E. creticum* Lam., *E. bungei* Bioss., *E. billardieri* F., *E. bourgatii* and *E. corniculatum* Lam. that chemical composition of these plants showed a lot of variations [22-25].

This information highlights the vast variations of the composition of these oils, perhaps due to interaction among genetic and environmental agents. These variations can be attributed to many factors such as climatic, geographical and seasonal variations. Some factors like nutrition, temperature, humidity, solar radiation and also genetics can be effective on chemical composition of the essential oils, as reported in the studies done by many researchers [26, 27]. In this research, while both kinds of these herbs grow in the same area and even at the similar altitude, their growing conditions, like the composition of the soil, are not totally the same. In addition, sample-taking may create further variability in oils. On the other hand, in our investigation the differences in the quantity of the constituents may be attributed to fertilization, since the wild populations do not have fertilization or irrigation, the wild herb depends on the rain in their water requirements. Generally, the diversity in essential oil composition among both cultivated and wild samples may be dependent on the more variable environment in wild growing situations as presented in many articles [28, 29]. According to the data of Table 1, it was deduced that the essential oil ingredients was not completely dependent on the herb whether wild or cultivated. The results demonstrated that principal components were same to the wild and cultivated samples [30-32]. This research also displayed that domestication only seems to influence the essential oil yields, not affecting the main essential oil ingredients that sound to be constant Results of this research illustrated that *E. caeruleum* had good adaptability for culture and domestication in Babol climatic situation. We will see the results of microbial tests also confirmed this statement. The tests of antimicrobial properties of the essential oils contained four strains of bacteria and two fungi. The MICs were specified as the lowest concentration of essential oil that inhibited the growth of a microorganism by the micro-dilution method. All the essential oils analysed were tested to contrast their antimicrobial properties (see Table 2).

Table 1 The main constituents of the essential oil of cultivated and wild *E. caeruleum* M.Bieb. plants grown in Babol area, Mazandaran, Iran.

No	Compound	RT	KI[a]	KI[b]	Cultivated%	Wild%	No	Compound	RT	KI[a]	KI[b]	Cultivated%	Wild%
1	α-Pinene	4.16	930	932	tr[c]	2.9	17	Cubebol	17.94	1514	1513	tr	1.6
2	β-Pinene	4.83	973	974	tr	1.8	18	β-Sesquiphellandrene	18.06	1521	1521	2.4	2.9
3	Myrcene	5.12	986	988	tr	1.7	19	Fluorene	19.89	1598	1594	tr	3.0
4	δ-3-Carene	5.55	1005	1008	9.5	6.6	20	β-Bisabolol	21.63	1674	1674	17.3	10.3
5	Limonene	6.06	1022	1024	16.4	32.3	21	(Z)-Apritone	21.87	1691	1689	tr	1.4
6	Linalool	7.51	1098	1095	tr	1.3	23	Amorpha-4,9-dien-2-ol	22.38	1704	1700	1.1	tr
7	(2E,4E)-Octadienol	8.04	1116	1113	1.9	tr	24	Phytol	30.24	1942	2118	2.9	1.8
8	Trans-p-Mentha-2,8-dien-1-ol	8.23	1117	1119	1.9	tr	25	trans- Ferruginol	33.87	2334	2331	1.0	tr
9	Cis-p-Mentha-2,8-Dien-1-ol	8.40	1129	1133	1.6	1.1	26	Totarolone	38.08	2539	2542	1.4	tr
10	Trans-Carveol	10.51	1218	1215	tr	1.2	27	Hinokienone	38.41	2561	2557	1.4	tr
11	Cis-Carveol	10.54	1224	1226	1.4	tr		Monoterpene Hydrocarbons				21.1	35.0
12	Carvone	11.11	1239	1239	2.8	1.0		Oxygenated Monoterpenes				10.4	11.0
13	δ-muurolene	13.62	1332	1336	7.8	3.1		Sesquiterpene Hydrocarbons				16.8	10.0
14	(E)-β-Farnesene	16.42	1459	1454	4.4	4.8		Oxygenated Sesquiterpenes				19.1	14.6
15	β-Bisabolene	17.70	1505	1505	1.5	tr		Total				81.3	78.8
16	γ-Cadinene	17.92	1513	1512	1.1	tr							

aRetention Index (RI) calculated using n-alkane series from C6 to C24 confirmed by comparison on DB-5MS; bRetention Index (RI) from literature data; ctr = relative percentage less than 1%.

Table 2 Antimicrobial activities of wild and cultivated *E. caeruleum* M.Bieb. (mg/ml)

		Staphylococcus aureus	Enterococcus faecium	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Candida Krusei
Wild sample	MIC	16	64	8	8	4	1
	MBC	>64	>64	16	>64	8	2
Cultivated sample	MIC	8	64	4	8	1	1
	MBC	>64	>64	8	>64	2	0.5
Cefixime	MIC	1	4	4	8	-	-
	MBC	8	32	16	>64	-	-
Nystatin	MIC	-	-	-	-	0.0313	0.0078
	MBC	-	-	-	-	0.0313	0.0078

The antibacterial analysis displayed that both essential oils presented high activity versus all the tested Gram-negative bacteria in a range of MIC values from 4 to 8 mg/ml. The oil of the cultivated sample showed stronger antibacterial activity against the wild sample and the lowest minimum inhibitory concentration. Results of the *Escherichia coli* and *Pseudomonas aeruginosa* strains, for cultivated sample were excellent and they were as the Cefixime drug.

For the investigated Gram-positive bacteria, the effective concentrations were among 8-64 Ml/ml. The antifungal activity of *E. caeruleum* has been evaluated against *Candida albicans* and *Candida krusei*. The data displayed moderate antifungal activity against both strains (Table 2). It is concluded that the essential oil extracted from cultivated sample was more impressive against all the organisms examined. This result can be interpreted by the connection that may exist among the type of action of oils and their chemical component. However, more investigations are needed to further demonstrate the connection among

In this research, both wild and cultivated oils were active against *Pseudomonas aeruginosa* tested strain. The survey results are very marvellous because *Pseudomonas aeruginosa* is a very important opportunistic pathogen causing wide range of infections by multiple virulence factors. Development of resistance to antibiotics makes cure of *Pseudomonas* infections increasingly hard. Moreover, in contrast to other bacteria, *Pseudomonas aeruginosa* commonly exhibit endurance to inhibition by plant essential oils. This biological activity might be regarding to the mono and sesquiterpenic compounds which represented a large fraction of oils and these components are famous for their remarkable biological activities the chemical components of the oils and their modes of action versus the desired microorganisms.

Previous research on *Eryngium* species have demonstrated various positive biological activities of essential oils within this genus, essentially antibacterial and antifungal properties. *E. tricuspidatum* displayed marvellous antibacterial and anticandidal properties with MIC values ranging from 4.6 to 74 Ml/ml [33].

Other research related to antibacterial properties of essential oils relying only on evaluation of inhibition diameter by disc diffusion manner. The oils of *E. creticum, E. campestre* and *E. thorifolium* (30 µL/disc) were additionally found to be effective against various methicillin-resistant *Staphylococcus aureus* strains and illustrated inhibition zones with diameters of 5-19 mm [34].

The volatile extracts of *E. duriaei* displayed antifungal properties with MIC values of 0.2-0.3 µL /ml versus various dermatophytes [35]. The results of these reports are proving that the *Eryngium* species have shown good biological activities.

[36]. In addition, the chemical components of these oils illustrate the predominance of oxygen-containing terpenoids in aerial parts of both samples. Many researches have proved that biological properties of oxygenated terpenes seem to exhibit higher potential than hydrocarbon ones [37], actually mono and sesquiterpenoids components may promote a broad spectrum of human health benefits, especially anti-inflammatory, antimicrobial and antitumor properties [38], this may illustrate the strong antibacterial effects on the microorganisms examined.

CONCLUSIONS

The results have been demonstrated that even though the numbers of *E. caeruleum* ingredients were dissimilar in wild and cultivated specimens but principal components were similar. The founded principal components were limonene, β-bisabolol and δ-3-carene. The biological evaluation in this research suggests that the essential oil of this herb exhibited a potent broad spectrum antimicrobial activity. This study suggests that this plant essential oil could be a natural alternative to synthetic and chemical preservatives to enhance food safety and life time. Our results, in addition, have been proved that cultivation and domestication of this plant seems improved the microbial test results for the cultivated sample [39, 40]. These are aspects that turn this plant into useful crops for domestication and commercialization [41]. Results of this investigation propose that it is noteworthy to grow this herb by the farmers in Babol, Mazandaran, Iran for pharmaceutical, therapeutic and food targets.

REFERENCES

1. Fayed E.M., Abd-EIGawad A.M., Elshamy A.I., El-Halawany E.S., EI-Amier Y.A. Essential oil of *Deverra tortuosa* aerial parts: Detailed chemical profile, allelopathic, antimicrobial, and antioxidant activities. Chem. Biodivers. 2021; 18(4): 441-452.
2. Kazemeini F., Asri Y., Mostafavi G., Kalvandi R., Mehregan I. Assessment of genetic diversity, population structure and morphological analyses in an Iranian endemic species *Rhabdosciadium aucheri* Boiss.(Apiaceae) using ISSR markers. Biologia. 2021; 76:441-51.
3. Sonboli A., Mirzania F., Gholipour A. Essential oil composition of *Dracocephalum kotschyi* Boiss. from Iran. Nat. Prod. Res. 2019; 33(14):2095-8.
4. Mozaffarian V. Umbelliferae. In: Flora of Iran. 2007. Research Institute of Forests and Rangelands, Tehran.
5. Parveen I., Techen N., Khan I.A. Identification of species in the aromatic spice family Apiaceae using DNA mini-barcodes. Planta Med. 2019; 85(02):139-44.
6. Mohammed M.S., Tawfik M.S., Ibrahim A.E. Influence of two extraction methods on essential oils of some Apiaceae family plants. Egypt. Pharm. J. 2019; 18(2):160.
7. Zengin G., Mahomoodally M.F., Paksoy M.Y., Picot-Allain C, Glamocilja J, Sokovic M, Diuzheva A, Jekő J, Cziáky Z., Rodrigues M.J., Sinan K.I. Phytochemical characterization and bioactivities of five Apiaceae species: Natural sources for novel ingredients. Ind. Crops Prod. 2019; 135:107-21.
8. Wang P., Su Z., Yuan W., Deng G., Li S. Phytochemical constituents and pharmacological activities of *Eryngium* L.(Apiaceae). Pharmaceutical Crops. 2012, 3: 99-120.
9. Gaudeul M., Till-Bottraud I., Barjon F., Manel S. Genetic diversity and differentiation in *Eryngium alpinum* L.(Apiaceae): comparison of AFLP and microsatellite markers. Heredity. 2004; 92(6):508-18.
10. Calvino C.I., Martínez S.G., Downie S.R. The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): Rapid radiations, long distance dispersals, and hybridizations. Mol. Phylogenet. Evol. 2008; 46(3):1129-50.
11. Benmerache A., Magid A.A., Berrehal D., Kabouche A., Voutquenne-Nazabadioko L., Messaili S., Abedini A., Harakat D., Kabouche Z. Chemical composition, antibacterial, antioxidant and tyrosinase inhibitory activities of glycosides from aerial parts of *Eryngium tricuspidatum* L. Phytochem. Lett. 2016; 18:23-8.
12. Ghahreman A. Flora of Iran. 1997, Recearch Institute of Forests and Rangelands, Tehran.
13. Khoshbakht K., Hammer K., Pistrick K. *Eryngium caucasicum* Trautv. cultivated as a vegetable in the Elburz Mountains (Northern Iran). Genet. Resour. Crop Evol. 2007; 54:445-8.
14. Riekandeh S.M., Mazandarani M., Ebrahimzadeh M.A., Zargari M. Antioxidant activities of *Eryngium caucasicum* inflorescence. Eur. Rev. Med. Pharmacol. Sci. 2016; 20(5):946-9.
15. Ebrahimzadeh M.A., Chitsaz Z., Shokrzadeh M., Ataie A., Ataee R. Evaluation of anticonvulsant activities of *eryngium caucasicum* with maximal electroshock and kindling model of seizure in mice. IJPBS. 2017; 11(1): 1-5.
16. Morteza-Semnani K. Essential oil composition of *Eryngium bungei* Boiss. J. Essent. Oil Res. 2005; 17(5):485-6.
17. Adams, P.R. Identification of Essential Oils Components by Gas Chromatography/ Quadrupole Mass Spectroscopy, 2007, Allured Publishing, Carol Stream, United States.
18. Jorgensen J.H., Turnidge J.D. Susceptibility test methods: Dilution and disk diffusion methods, DC: Manual of Clinical Microbiology, 2007, ASM Press, Washington.
19. Ana V., Espinel-Ingroff, A.V. and Pfaller, M.A. Susceptibility Test Methods: Yeasts and Filamentous Fungi, Manual of Clinical Microbiology, 2007, ASM Press, Washington.
20. Hashemabadi D., Kaviani B., Erfatpour M., Larijani K. Comparison of essential oils compositions of eryngo (*Eryngium caucasicum* Trautv.) at different growth phases by hydrodistillation method. Plant Omics. 2010; 3(4):135-9.
21. Hashemabadi D., Kaviani B. Chemical constituents of essential oils extracted from the leaves and stems of

Eryngium caucasicum Trautv. from Iran. J. Essent. Oil-Bear. Plants. 2011; 14(6):693-8.
22. Tohidi B., Rahimmalek M., Arzani A. Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of *Thymus* species collected from different regions of Iran. Food chem. 2017; 220:153-61.
23. Sefidkon F., Dabiri M., Alamshahi A. Chemical composition of the essential oil of *Eryngium billardieri* F. Delaroche from Iran. J. Essent. Oil Res. 2004; 16(1):42-3.
24. Palá-Paúl J., Perez-Alonso M.J., Velasco-Negueruela A., Vadaré J., Villa A.M., Sanz J., Brophy J.J. Essential oil composition of the different parts of *Eryngium bourgatii* Gouan from Spain. Journal of Chromatography A. 2005; 1074(1-2):235-9.
25. Palá-Paúl J., Brophy J.J., Pérez-Alonso M.J., Usano J., Soria S.C. Essential oil composition of the different parts of *Eryngium corniculatum* Lam.(Apiaceae) from Spain. J. Chromatogr. A. 2007; 1175(2):289-93.
26. Mirzania F., Sarrafi Y., Farimani M.M. Comparison of chemical composition, antifungal antibacterial activities of two populations of *Salvia macilenta* Boiss. Essential oil. Rec. Nat. Prod. 2018; 12: 385-90.
27. Mirzania F., Sarrafi Y., Moridi Farimani M. Comparative evaluation of chemical compositions and biological activities of wild and cultivated *Froriepia subpinnata* L. essential oils. JAST. 2019; 21(2):331-40.
28. Tock M.L., Kamatou G.P., Combrinck S., Sandasi M., Viljoen A.M. A chemometric assessment of essential oil variation of three *Salvia* species indigenous to South Africa. Phytochem. 2020; 172:112249.
29. Mirzania F., Moridi Farimani M., Sarrafi Y., Nejad Ebrahimi S., Troppmair J., Kwiatkowski M., Stuppner H., Alilou M. New Sesterterpenoids from *Salvia mirzayanii* Rech. f. and Esfand. Stereochemical Characterization by Computational Electronic Circular Dichroism. Front. Chem. 2022, 20; 9:1166.
30. Pirbalouti A.G., Hashemi M., Ghahfarokhi F.T. Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. Ind. Crops Prod. 2013; 48: 43-8.
31. Mirzania F., Farimani M.M. Biochemical evaluation of antioxidant activity, phenol and flavonoid contents of *dracocephalum kotschyi* boiss extracts obtained with different solvents. HBB. 2018; 1:32-44.
32. Merghache D., Boucherit-Otmani Z., Merghache S., Chikhi I., Selles C., Boucherit K. Chemical composition, antibacterial, antifungal and antioxidant activities of Algerian *Eryngium tricuspidatum* L. essential oil. Nat. Prod. Res. 2014; 28(11):795-807.
33. Çelik A., Aydınlık N., Arslan I. Phytochemical constituents and inhibitory activity towards methicillin-resistant *Staphylococcus aureus* strains of *Eryngium* species (Apiaceae). Chem. Biodivers. 2011; 8(3):454-9.
34. Erdem S.A., Nabavi S.F., Orhan I.E., Daglia M., Izadi M., Nabavi S.M. Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus *Eryngium*. DARU J. Pharm. Sci. 2015; 23:1-22.
35. Cavaleiro C., Gonçalves M.J., Serra D., Santoro G., Tomi F., Bighelli A., Salgueiro L., Casanova J. Composition of a volatile extract of *Eryngium duriaei* subsp. juresianum (M. Laínz) M. Laínz, signalised by the antifungal activity. JPBA. 2011; 54(3):619-22.
36. Merghache D., Boucherit-Otmani Z., Merghache S., Chikhi I., Selles C., Boucherit K. Chemical composition, antibacterial, antifungal and antioxidant activities of Algerian *Eryngium tricuspidatum* L. essential oil. Nat. Prod. Res. 2014; 28(11):795-807.
37. Çelik A., Aydınlık N., Arslan I. Phytochemical constituents and inhibitory activity towards methicillin-resistant *Staphylococcus aureus* strains of *Eryngium* species (Apiaceae). Chem. Biodivers. 2011; 8(3):454-9.
38. Guo R., Duan Z.K., Li Q., Yao G.D., Song S.J., Huang X.X. Guide isolation of guaiane-type sesquiterpenoids from *Daphne tangutica* maxim. And their anti-inflammatory activities. Phytochem. 2023; 206:113523.
39. Sefidkon F., Adbollahi M., Salehi Shanjani P., Tavakoli M. Variability in Essential Oil Content and Composition of *Achillea tenuifolia* Lam. Populations in Field Conditions. JAST. 2021; 23(3):673-83.
40. Canan S., Ceyhan V. Exploring the farm level opportunity cost for protecting environment: evidence from Turkey. JAST. 2021; 23(2):253-63.
41. Riahi Rad K., Babaei A., Mozaffarian V., Potter D. Phylogenetic affinities of wild and cultivated *Ornithogaloideae* based on ITS and trnL–F DNA sequences by extended sampling from Iran. JAST. 2019; 21(4):1005-21.