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Comparison of essential oils compositions of eryngo (*Eryngium caucasicum* Trautv.) at different growth phases by hydrodistillation method

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Abstract

The essential oils extracted from stems and leaves of *Eryngium caucasicum* Trautv., by hydrodistillation method. The samples were collected from different coastal and hill slope locations at various vegetative phases. The composition of essential oils was analysed by gas chromatography (GC) and gas chromatography, coupled to mass spectrometry (GC-MS). Totally, fifty eight volatile compounds were identified in stem and leave oils. Quantitative and qualitative differences were also found among the analysed parts. The β-sesquiphellandrene (44.21%), limonene (18.39%) and β-bisabolene (6.08%) were dominant components, respectively, at vegetative phase (May 2009) in the leaves of coastal plants, while 5-methyl-2-pyrimidone (53.83%), β-sesquiphellandrene (11.26%) and β-bisabolene (7.43%) were dominant components in hill slope plants. The main components of the essential oils in leaves of coastal plants at vegetative phase (June 2009) were β-sesquiphellandrene (27.32%), limonene (14.32%) and 5-methyl-2-pyrimidone (14.15%). On the hill slope, plant components were 4(5)-acetyl-1H-imidazole (50.14%), β-sesquiphellandrene (15.51%) and 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (11.05%). The Hexadecahydro-cyclobuta [1,2,3,4] dicyclooctene (45.46%), β-sesquiphellandrene (20.5%) and widdrene (19.06%) were dominant components at generative phase (July 2009) in the stems of coastal plants, while piperiton (69.81%), 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (18.38%) and β-sesquiphellandrene (4.54%) were dominant components on hill slope plants. The main components of the essential oils were sesquiterpenes. This study shows that type and concentration of components can be remarkably changed, based on vegetation phase and location. This can help us to be more selective in our extraction strategy.

Keywords: *Eryngium caucasicum* Trautv., Apiaceae, Essential oil, Hydrodistillation, β-Sesquiphellandrene

Introduction

Eryngium caucasicum Trautv. (Apiaceae) is a perennial herbaceous plant with about one meter height, an endemic species that has been distributed in the northern parts of Iran (Ghahreman, 1997). The plant leaves are normally used in medicine and food industries in Iran (Khoshbakht et al., 2006). *Eryngium caucasicum* Trautv. has several medicinal properties including enforcing generative power, diuretic, lenitive and appetizer (Semnani et al., 2003; Trautv, 2004). The essential oils have already been extracted from the various parts (roots, stems, leaves and inflorescences) of eryngo (Palá-Paúl et al., 2007; Palá-Paúl et al., 2005; Capetanos et al., 2007; Cobos et al., 2002; Cardozo et al., 2004; Martins et al., 2003; Pino et al., 1997; Wong et al., 1994; Brophy et al., 2003). It is well-known that essential oil components are biosynthesized in the plants as secondary metabolites; therefore their composition is highly variable and depends on several factors, such as climatic conditions, harvesting time, plant cultivar and plant chemotype (Bylaitė et al., 1998; Bylaitė et al., 2000; Santos-Gomes and Fernandes-Ferreira, 2001). The essential oils of plants have usually been isolated by either hydrodistillation or solvent extraction. Variations in the yield and the composition of essential oil at different vegetation phases and different climatic conditions have already been reported in some species (Bylaitė et al., 2000). Thus, the purpose of this study was to assess essential oils content and its composition of eryngo in vegetative and generative stages harvested from coastal and hill slope regions in the northern parts of Iran.

Materials and methods

Plant materials

Aerial parts (leaves and stems) of eryngo (*Eryngium caucasicum* Trautv.) were harvested on 5th May, June and July 2009 which are first, second vegetative and generative phase of plant growth, respectively.

Collection area

Plants were collected from coastal (-28 m altitude) and hill slope (213 m altitude) regions of Langeroud city, east of Guilan province, in the north of Iran.

Oil extraction and GC-MS analysis

The plant materials were dried in 45°C and kept air-tight in 2°C till extraction. The 30 g dried plant materials were subjected to hydrodistillation for 4 h using a Clevenger- type apparatus to produce volatiles. The essence were dried over anhydrous calcium chloride and stored in sealed vials at 2°C before analysis.

Table 1. Essential oil compounds in first vegetative phase obtained from *Eryngium* leaves of coastal area

No.	Compounds	Amount (%)	Retention time (min)
1	Octane	3.11	5.04
2	Δ-3-Carene	1.76	9.79
3	Limonene	18.39	10.18
4	1-Menthone	3.88	13.07
5	(+)- Pulegone	5.45	14.93
6	(-)- endo-2,6-dimethyl-6-(4-methyl-3-pentenyl) bicycl	2.16	18.79
7	Trans-β-farnesene	2.89	18.94
8	(-)-5-Epiprezizaene	1.45	19.40
9	1-(1,5-dimethyl-4-hexenyl)-4-methyl-benzene	1.67	19.61
10	β-Ionone	2.53	19.75
11	β–Cubebene	5.08	19.89
12	β–Bisabolene	6.08	20.08
13	β–Sesquiphellandrene	44.21	20.40
14	Δ-Cadinene	1.35	20.49

Table 2. Essential oil compounds in first vegetative phase obtained from *Eryngium* leaves of hill slope area

No.	Compounds	Amount (%)	Retention time (min)
1	Octane	0.33	4.94
2	Octanal	0.51	9.05
3	1-Limonene	5.62	9.79
4	α-Cedrol	1.79	13.14
5	Carvone	0.89	14.30
6	Norsesterterpene Dien Ester	1.27	14.59
7	1(7), 5, 8-O-Menthatriene	0.89	14.88
8	β-Bisabloene	0.69	15.89
9	Gamma-dodecalactone	1.24	16.57
10	5-Methyl-2-Primidone	53.83	16.83
11	4-(1,5-Dimethyhex-4-enyl)cyclohex-2-enone	6.08	17.31
12	CIS-caryophyllene	0.83	17.80
13	Trans-β-farnesene	1.79	17.99
14	Widdrene	0.79	18.12
15	1(2H)-Naphtalonene,3,4-dihydro	1.01	18.23
16	(E,Z)-Alpha-Farnesene	0.62	18.45
17	1-(1,5-Dimethyl-4-hexenyl-4-methyl-benzene	0.76	18.63
18	β-Ionene	1.01	18.76
19	β-Bisabolene	7.43	19.06
20	β - Sesquiphellandrene	11.26	19.36
21	3- Methyl-1,2-bezisothiazole	1.42	19.56

GC-MS analysis were carried out on a GC-HP-6890 with HP-5MS (30 m, 0.35 mm (i.d.). Oven temperature was at 60°C for 3 min and increased to 220°C at the rate of 7°C min^{-1}. The gas speed in column was 1 $mm.min^{-1}$ and the type of carrier gas was He (99.999%). The components of the essential oils were identified by comparison of their mass spectra with those of computer library and confirmed by comparison their retention indices with data published in the literature.

Results

Fourteen and twenty-one compounds were detected in the leaves essential oils obtained from coastal and hill slope samples, in the first vegetative phase, respectively (Tables 1 and 2). In this phase and in coastal samples, β-sesquiphellandrene (44.21%), limonene (18.39%) and β-bisabolene (6.08%) were dominant components, while 5-methyl-2-pyrimidone (53.83%), β-sesquiphellandrene (11.26%) and β-bisabolene (7.43%) were dominant components in hill slope. The content of three major compounds obtained from first vegetative phase in coastal and hill slope regions were 68.68% and 72.52%, respectively.

In second vegetative phase, twenty-four and nine compounds were identified in the leaves essential oils of samples collected from coastal and hill slope samples, respectively (Tables 3 and 4). The main components of the essential oils in the leaves of coastal plants were β-sesquiphellandrene (27.32%), limonene (14.32%) and 5-methyl-2-pyrimidone (14.15%), and in hill slope plants were 4(5)-acetyl-1H-imidazole (50.14%), β-sesquiphellandrene (15.51%) and 4-(1,5-dimethylhex-4-enyl)cyclohex-2-enone (11.05%). Three main compounds extracted from the second vegetative phase in coastal and hill slope areas contained 55.79% and 76.70%, respectively.

Among 15 and 7 compounds extracted from the stems of coastal and hill slope at generative phase, the major components in coastal plants were hexadecahydro-cyclobuta [1,2:3,4] dicyclooctene (45.46%), β-sesquiphellandrene (20.50%) and widdrene (19.06%), while piperiton (69.81%), 4-(1,5-dimethylhex-4-enyl) cyclohex-2-enone (18.38%) and β-sesquiphellandrene (4.54%) were dominant components in hill slope plants (Tables 5 and 6). Three principal compounds of this phase in coastal and hill slope areas included 85.02% and 92.73%, respectively.

The main compound of the essential oils extracted from stems and leaves of *Eryngium caucasicum* Trautv. were sesquiterpenes and the major component present in both vegetative phase and generative phase from coastal and hill slope regions was β-sesquiphellandrene. In generative phase, eryngo is not edible.

Table 3. Essential oil compounds in second vegetative phase obtained from *Eryngium* leaves of coastal area

No.	Compounds	Amount (%)	Retention time (min)
1	Octane	2.44	5.04
2	α-Pinene	0.68	8.08
3	Myrcene	0.91	9.14
4	Δ-3-Carene	0.90	9.79
5	1-Limonene	14.32	10.19
6	Ttans-para-2,8-menthadien-1-ol	0.46	12.26
7	1-Ethyl-3-isopropylbenzene	0.70	14.34
8	L-Carvone	0.82	14.99
9	CIS-Piperiton oxide	0.64	15.18
10	Thymol	9.93	15.70
11	5-Methyl-2-pyrimidone	14.15	16.98
12	Piperitone oxide	2.04	17.51
13	β-Elemene	1.52	18.03
14	5-Acetyl-2-hydrazino-4-methylpyrimidine	1.02	18.27
15	Trans-Caryophyllene	3.84	18.78
16	Trans-β-Farnesene	3.28	18.95
17	CIS-α-bisabolene	1.90	19.41
18	(-)-AR-Curcumene	1.29	19.62
19	β-Ionone	1.29	19.75
20	Germacrened	2.63	19.89
21	β-Bisabolene	6.45	20.08
22	α-Selinene	0.81	20.16
23	β-Sesquiphellandrene	27.32	20.42
24	Δ-Cadinene	0.64	20.50

Table 4. Essential oil compounds in second vegetative phase obtained from *Eryngium* leaves of hill slope area

No.	Compounds	Amount (%)	Retention time (min)
1	Bornylene	4.33	9.79
2	4(5)-Acetyl-1H-imidazole	50.14	16.72
3	4-(1,5-Dimethylhex-4-enyl)cyclohex-2-enone	11.05	17.25
4	1,3,5,7-Tetramenthyladamantane	0.84	18.76
5	1-Isopropyl-3-tert-butylbenzene	1.06	18.89
6	β-Bisabolene	1.31	19.06
7	1,2-Benzenedicarboxylic acid	7.69	19.26
8	β-Sesquiphellandrene	15.51	19.36
9	8-Quinolinemetanol	0.87	19.54

Discussion

The results show that the compounds of essential oils is very environmental-dependent which means a variety of plants obtained from different regions have different compounds. In our study, highly significant qualitative and quantitative differences and similarities were observed among the samples obtained from vegetative and generative phases in coastal and hill slope plants. In coastal samples, the quantity of essential oil components was greater than that on the hill slope samples. The essential oils of *Eryngium* have shown different compositions in previous studies. In agreement with this study, Capetanos et al. (2007) showed that the essential oils were complex mixture of fifty eight different compounds in each investigated case on two *Eryngium* species (*E. palmatum* and *E. serbicum*). On the contrary with our study, the contribution of the main compounds never exceeding 20% of the total. Among fifty eight different compounds obtained by these researchers, sesquiterpenes were the main constituents in both studied species which conforming our data. The main constituents of the investigated *Eryngium* essential oils, reported by Capetanos et al. (2007), were germacrene D (19.7%), β-elemene (10.0%) and spathulenol (6.9%) in *E. serbicum*, and sesquicineole (21.3%), caryophyllene oxide (16.0%), spathulenol (6.6%) and sabinene (4.4%) in *E. palmatum*. Also, Brophy et al. (2003) revealed that the major compounds of the essential oils were bornylacetate (20.8%), selinene (13.8%), α-selinene (11.3%) and α-muurolene (8%) in *E. pandanifolium*, and spathulenol (20%) and β-bisabolol (8.6%) in *E. rostranum*. Martins et al. (2001) extracted the essential oils of two samples of *Eryngium foetidum* from different regions and observed that 2,3,6- trimethylbenzaldehyde (5.5 to 23.7%), (E)-2-dodecenal (15.9 to 37.5%) and (E)-2-tetracenal (18.7 to 25.3%) were the most important components. In this study, 24 compounds were identified with significant similarities in quality but there were some quantitative differences. For example, 2,3,6-trimethylbenzaldhyde (23.7%) was the most important in first sample, while in second sample, (E)-2-dodecenal (37.5%) was significant compound. In the essential oils of *E. paniculatum*, (E)-anethole (52.6%) was found as major component (Cobos et al., 2002), while in *E.billardieri,*α-muurolene was dominant (Sefidkon et al., 2004).

Several investigations of the essential oils of *E. foetidum* revealed that fatty acids and aldehydes were the main constituents (Martins et al., 2001; Wong et al., 1994; Leclercq et al., 1992). Wong et al. (1994) reported (E)-2-dodecenal (59.7%) in the leaves and 2,3,6-trimethylbenzaldhyde (37.6%) in the roots were dominant. Leclercq et al. (1992) found (E)-2-dodecenal (45.5%), dodecanoic acid (15.5%) and 2-dodecenal acid (8.6%) as the major compounds. According to the Capetanos et al. (2007) results, the essential oils of *E. palmatum and E. serbicum* have several differences, as their main components differ significantly.

Table 5. Essential oil compounds in generative phase obtained from *Eryngium* stems of coastal area

No.	Compounds	Amount (%)	Retention time (min)
1	Octane	3.17	0.05
2	Octanal	0.77	9.40
3	Limonene	8.37	10.19
4	Thymol	1.44	15.69
5	Hexadecahydro-cyclobuta[1,2,3,4]dicyclooctene	45.46	17.07
6	2,4-Bis(1,1-dimethyethyl)-phenol	4.16	17.65
7	Isocaryophyllene	1.79	18.78
8	Trans-β-farnesene	2.61	18.94
9	Widdrene	19.06	18.96
10	(E,Z)-α-Farnesene	1.07	19.41
11	1-(1,5-Dimethyl-4-hexenyl)-4-methyl-benzene	1.25	19.62
12	Ionone	1.02	19.75
13	β-Bisabolene	6.25	20.08
14	β-Sesquiphellandrene	20.5	20.41
15	3,4-Dimethyl-pyridine	1.22	21.34

Table 6. Essential oil compounds in generative phase obtained from *Eryngium* stems of hill slope area

No.	Compounds	Amount (%)	Retention time (min)
1	Thymol	1.67	14.96
2	Piperiton	69.81	16.80
3	4-(1,5-Dimethylhex-4-enyl)cyclohex-2-enone	18.38	17.30
4	2(3-Butanone)-1-tetralone	1.37	18.22
5	β-Bisabolene	2.27	19.06
6	β-Sesquiphellandrene	4.54	19.37
7	3a-Hydroxy-3,6,8-trimethyl-2,3,3a,8b-tetrahydro-1H	1.97	19.54

Cardozo et al. (2004) revealed 2,4,5-trimethylbenzaldehyde (27.7%) and (E)-2-dodecenal (27.5%) as the dominant components. In contrast, Pino et al. (1997) reported that caryophyllene oxide (19.3%) was the main compounds.

Current study confirmed that the three important factors effective on quantitative and qualitative characters of the essential oils are highly associated with geographical position, extraction time and plant species. In this study, comparison of the essential oils obtained from *E.caucasicum* has shown only one (limonene, 8%) and two (limonene, 52.1% and β-sesquiphellandrene, 8.1%) similar compounds, respectively (Semnani et al., 2003).

There are some differences and also similarities between the essential oils, obtained from different species and cultivars (Khajeh et al., 2005; Dvaranauskaite et al., 2008; Santos et al., 2005). It is notable that variations in the total essential oil contents and in the concentrations of individual components were observed for the studied *Eryngium* and other species at different development phase. Studies of Dvaranauskaitė et al. (2008), on essential oil composition in buds of six *Ribes nigrum* L. cultivars at difference development phases, showed that the concentration of the main oil compounds in buds, harvested in April, was 2-50 times lower than other periods in all six cultivars. Variations in essential oils strongly depend on the genetic peculiarities, different geographical places and harvesting season.

Consequently, individual approach should be applied to every individual plant species in order to select an optimal time of harvesting for the highest product quality and economical value. In generative phase, eryngo (*Eryngium caucasicum* Trautv.) is not edible. Therefore, the suitable time for harvesting eryngo is the beginning of May.

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