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Essential oil constituents and fatty acids in *Echium amoenum* grown wild in Iran

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Abstract

This study was led with the purpose of evaluating the essential oil constituents of flower and Fatty acids in seeds of *Echium amoenum* grown wild in Iran. Flower essential oil was obtained by hydro-distillation using Clevenger type apparatus during approximately 3 hours and analyzed using gas chromatography/mass spectrometry (GC/MS). Fifty three components were identified in *E. amoenum* flowers. The main components of the oil were n-Tricosane (33.92%), n-Pentacosane (21.09%), Palmitic acid (18.91%), Linoleic acid (3.51%) and Pulegone (2.20%). The oil of seeds was extracted by soxhlet and analyzed using gas chromatography/mass spectrometry. Twenty one fatty acids were identified in *E. amoenum* seed oil that the main components were, Linoleic Acid (c18:3, Alpha) (33.64%), Linoleic Acid (c18:2,c-c) (21.25%), Oleic Acid (c18:1,c) (17.94%), Palmitic Acid (c16:0) (7.82%), Gadoleic Acid (c20:1) (6.78%), Linoleic Acid (c18:3,Gamma) (5.88%) and Steric Acid (c18:0) (5.14%).

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Introduction

Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents (Nychas *et al.*, 2003). *Echium* genus has 4 species in Iran, including *Echium amoenum*, *E. russicum*, *E. italicum* and *E. khuzistanicum* (Ghassemi *et al.*, 2003). *Echium amoenum* (Iranian borage) is a biennial or perennial plant with bright blue flower and fruit comprise of four brownish-black nut lets, from Boraginaceae family. It is native of Iran and has been seen in the northern parts of Iran in Alborz Mountains and some parts of these areas in the altitudes between 1500 to 2000 m from sea level (Ebrahimi, 2012).

Echium amoenum is one of the important medicinal herbs in traditional Iranian medicine. In Iranian traditional medicine, the flowers of *E. amoenum* were used by the people to cure the symptoms of cold and as an agent to increase the blood pressure, antidepressant, and poultice for inflammatory, relaxer, diuretic and perspire (Noorhosseini-niyaki, 2013 and Zargari, 1990). Recent studies on phytochemical constituents of *E. amoenum* revealed the presence of many chemicals such as flavonoids, saponins, fatty acids and unsaturated terpenoids and sterols (Shafaghi, 2002). Literature review on essential oil composition of *E. amoenum* indicate that, δ -cadinene (24.25%) as the major component of this essential oil (Ghassemi *et al.*, 2003). Also the flower of this plant have anthocyanidine (13%), flavonoid aglycons (0.15%) and trace amount of alkaloids (Mehrabani *et al.*, 2005). The seeds of *E. amoenum* consisted many fatty acids such as linoleic acid and Linolenic acid as omega-3 that these fatty acids have biomedical importance for body (Babakhanzadeh Sajirani *et al.*, 2014).

The aim of this study was to determine the essential oil composition in flowers and fatty acids in seeds of *E. amoenum* grown wild in Iran as possible new source for valuable components and fatty acids for use in traditional and industrial medicine.

Materials and methods

Plant materials

Flowers and seed samples of *E. amoenum* were purchased from local markets. One hundred grams of each samples was crushed and ground in a household grinder.

Essential oil extraction

Essential oil was obtained from *E. amoenum* flowers (100 g) that were subjected to hydro-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oil drying by anhydrous sodium sulfate. The isolated oils were stored in tightly closed vials at 4°C until analysis.

Seed oil extraction

The finely ground seed of *E. amoenum* (10 g) was weighed in a glass and then macerated with Normal Hexane by sox let for 15 h for oil extraction. The oil extraction was filtrated.

Through whatman filter paper (Whatman Ltd., England). Supernatants were combined and evaporated to dryness using a rotary evaporator to a volume of about 1 ml in less than 40°C. One ml of hexane extract combined with 20 ml potassium dissolve in methanol for 25 minutes, and then 12 ml Fluoro bormide add to this extract. After 10 minutes, one gr Nacl was add in the extract, then the hexane phase that consist fatty acids was separated, these sample was stored for fatty acids experiment.

Essential oil and oil analysis

Essential oil and oil seed were analyzed by Hewlett – Packard GC/MS (model 7890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m' 0.25mm, 0.25 μ m film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method (Van den dool and Kratz, 1963). Using n–alkanes as standard. The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra

with the Wiley and Mass finder 3 libraries or with the published mass spectra (Adams, 2001).

Result and discussion

Essential oil constituents

The chemical composition of the essential oil of *E. amoenum* flowers (purchased from local markets from Iran) and the retention indices are presented in (Table 1). Fifty three components were identified in *E. amoenum* flowers that represented 99.75% of the oil. The main components of the oil were n-Tricosane (33.92%), n-Pentacosane (21.09%), Palmitic acid (18.91%), Linoleic acid (3.51%) and Pulegone

(2.20%). The information on the essential oil of *E. amoenum* is very scarce and limited. Ghassemi *et al.*, (2003) reported that δ -cadinene (24.25%), viridiflorol (4.9%), α -muurolene (4.52%), ledene (3.8%), α -calacorene (3.04%) and α -cadinene (2.9%) were the major components of *E. amoenum* essential oil. These Differences in the essential oil compositions in our research and previous study in this plant, can be attributed several factors such as climatic, seasonal and geographical or ontogenesis variations. The different parts of the plant analysis and the different methods of drying or essential oil extraction (Alizadeh *et al.*, 2013 and Alizadeh, 2013).

Table 1. Essential oil constituents of *Echium amoenum* analyzed by (GC/MS).

No	Compound	RI	Percentage of oil
1	Cyclohexanone	895	0.08
2	n-Octanal	1001	0.25
3	p-Mentha-3,8-diene	1066	0.09
4	2-Nonanone	1089	0.03
5	n-Undecane	1097	0.08
6	n-Nonanal	1102	0.54
7	Methyl glutarate	1134	0.14
8	(2E)-Nonen-1-al	1156	0.1
9	neo-Menthol	1161	0.11
10	n-Dodecane	1196	0.07
11	n-Decanal	1202	0.62
12	Pulegone	1236	2.20
13	Thymol	1290	0.81
14	1-Tridecene	1296	0.26
15	Carvacrol	1299	0.35
16	Undecanal	1304	0.28
17	2,4-Decadienal	1313	0.07
18	Piperitenone	1338	0.14
19	\square -Terpinyl acetate	1346	0.12
20	\square -Copaene	1372	0.06
21	\square -Bourbonene	1381	0.03
22	n-Tetradecane	1396	0.34
23	Dodecanal	1405	0.42
24	(E)-Caryophyllene	1416	0.16
25	Geranyl acetone	1450	0.06
26	ar-Curcumene	1480	0.10
27	n-Pentadecane	1496	0.39
28	Tridecanal	1507	0.42
29	Dodecanoic acid	1561	0.23
30	Spathulenol	1574	0.64
31	Caryophyllene oxide	1579	0.40
32	n-Hexadecane	1595	0.41
33	Tetradecanal	1608	0.79
34	ar-Turmerone	1662	0.21
35	n-Heptadecane	1695	0.27
36	Unknown	1710	0.85
37	Myristic acid	1760	1.17
38	n-Octadecane	1795	0.32
39	Hexadecanal	1811	0.39

40	6,10,14-trimethyl-2-	1839	0.27
41	Isobutyl phthalate	1861	0.35
42	1-Nonadecene	1891	0.27
43	Hexadecyloxirane	1909	0.36
44	Palmitic acid	1960	18.91
45	n-Eicosane	2000	0.53
46	n-Heneicosane	2100	0.97
47	Linoleic acid	2141	3.51
48	Stearic acid	2171	1.88
49	n-Docosane	2198	1.16
50	n-Tricosane	2302	33.92
51	n-Tetracosane	2400	1.92
52	n-Pentacosane	2504	21.09
53	Bis(2-ethyl hexyl) phthalate	2562	0.61
	Total		99.75

aRI, retention indices in elution order from HP – 5 column.

Table 2. Fatty acid components of *Echium amoenum* seed oil.

No	Components	RT (min)	Percentage of seed oil
1	Lauric Acid (c12:o)	17.58	0.06
2	Myristic Acid (c14:o)	20.84	0.05
3) Palmitic Acid (c16:o)	25.32	7.82
4	Palmitic Acid (c16:1,T)	26.98	0.04
5	Palmitic Acid (c16:1,c)	27.31	0.14
6	Margaric Acid (c17:o)	29.21	0.24
7	Steric Acid (c18:o)	34.28	5.14
8	Elaidic Acid (c18:1,T)	35.93	0.10
9	Oleic Acid (c18:1,c)	37.15	17.94
10	Linoleic Acid (c18:2,c-T)	40.78	0.18
11	Linoleic Acid (c18:2,c-c)	42.30	21.25
12	Linoleic Acid (c18:2,c)	43.22	0.06
13	Linoleic Acid (c18:2,c)	44.46	0.11
14	Linoleic Acid (c18:3,Gamma)	46.18	5.88
15	Linoleic Acid (c18:3,T)	48.27	0.10
16	Linoleic Acid (c18:3, Alpha)	49.77	33.64
17	Arachidic Acid (c20:o)	51.25	0.06
18	Gadoleic Acid (c20:1)	54.55	6.78
19	Behenic Acid (c22:o)	70.69	0.04
20	Erucic Acid (c22:1)	75.38	0.11
21	Lignoceric Acid (c24:o)	97.57	0.01
	Total		99.75

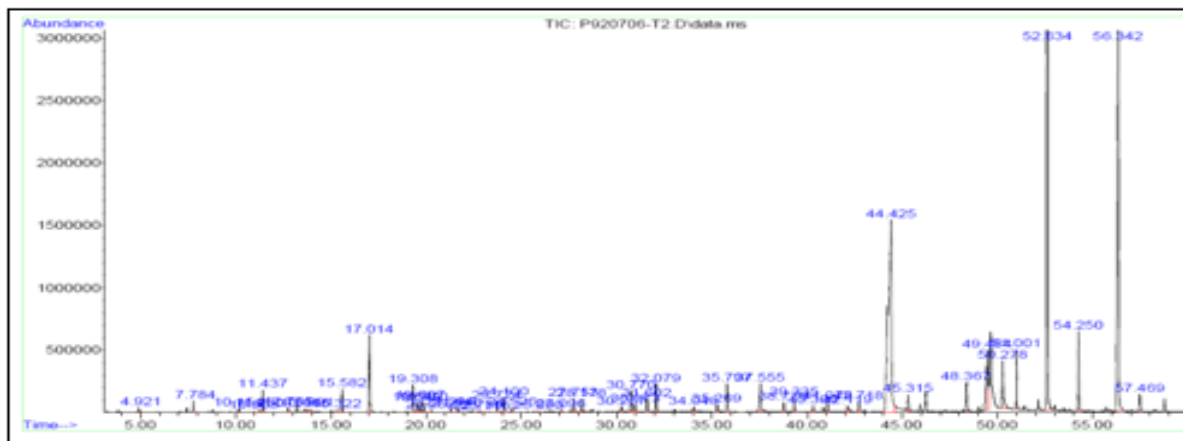
Fatty acids constituents

The fatty acids constituents of *E. amoenum* seed oil are presented in (Table 2). Twenty one fatty acids were identified in *E. amoenum* seed oil that represented 99.75% of the oil. The main fatty acids on the oil were, Linoleic Acid (c18:3, Alpha) (33.64%), Linoleic Acid (c18:2,c-c) (21.25%), Oleic Acid (c18:1,c) (17.94%), Palmitic Acid (c16:o) (7.82%), Gadoleic Acid (c20:1) (6.78%), Linoleic Acid (c18:3,Gamma) (5.88%) and Steric Acid (c18:o) (5.14%) that 13.47%

of this are saturated and 86.28% unsaturated fatty acids (Table 3). Babakhanzadeh Sajirani *et al.*, (2014) reported that α -Linolenic acid (35.69%), Linoleic acid (20.68%), and Oleic acid (17.08%) were the main fatty acids constituents in *E. amoenum* seed oil. The fatty acids identified in our research are higher than previous study in this plant but our results in fatty acids constituents are similar with previous study by Babakhanzadeh Sajirani *et al.*, (2014).

Table 3. Saturated and un saturated fatty acid percentage of *Echium amoenum* seed oil.

Saturated Fatty Acids	13.47%
Un Saturated Fatty Acids	86.28%

**Fig. 1.** Chromatogram of essential oil constituents of *Echium amoenum* flowers.

Conclusion

In conclusion, results of this study indicate that *E. amoenum* has valuable constituents and fatty acids in flowers essential oil and seeds oil. The results of this study was supported the utility of *E. amoenum* in folk and traditional medicine.

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