Journal of Medicinal Plants Research Vol. 5(19), pp. 4814-4821, 23 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Characterization of fatty acids in different organs of some Iranian *Echium* plants

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Accepted 22 June, 2011

Seeds, leaves and stems of six populations of two *Echium* species (Family Boraginaceae) from Iran were surveyed for the first time in a search for new sources of γ-linolenic acid (GLA, C18:3ω6), stearidonic acid (SDA, 18:4ω3) and other unsaturated fatty acids. Oils from the corresponding organs were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) for total oil content and fatty acid composition. The range of total oil content varied between 0.7% in stems of *Echium amoenum*, Behshahr population to 33.8% in seeds of *E. amoenum,* Ramsar population. The amounts of GLA ranged from 2.28% of total fatty acids in *E. amoenum* stems (Behshahr population) to 9.79% in *Echium italicum* seeds (Kaleybar population). In addition, considerable amounts of SDA were detected, with the highest values of 10.47 and 12.45% in seeds of *E. italicum* from Boumehen and Kaleybar populations, respectively. Other main unsaturated fatty acid components in the oils were α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LA, C18:2ω6) with the highest amounts of 44.15 and 26.94% in *E. amoenum* seeds from Hezar Jarib and Behshahr populations, respectively. This data allows us to evaluate Iranian members of *Echium* genus as alternative wild sources of valuable unsaturated fatty acids for commercial purposes.

Key words: Boraginaceae, *Echium,* fatty acids, γ-linolenic acid, stearidonic acid, gas chromatography.

INTRODUCTION

Polyunsaturated fatty acids with 18 carbons (C18 PUFA) exist in several fish and plant species, but the seeds of higher plants are the richest sources. This is because higher plants lack the metabolic pathways to produce other PUFA with 20 or more carbons, so that linoleic acid (LA, 18:2ω6), α-linolenic acid (ALA, 18:3ω3), γ-linolenic acid (GLA, 18:3ω6) and stearidonic acid (SDA, 18:4ω3) accumulate as final fatty acid products (López-Martínez et al., 2005; Guil-Guerrero et al., 2007). GLA is the first intermediate in the conversion of LA to arachidonic acid (ARA, 20:4ω6) by the enzyme Δ^6-desaturase. The Δ^6-desaturation is usually the rate-limiting step for the synthesis of the essential long-chain PUFAs (Gunstone, 1992). Under some conditions, this enzymatic activity is depressed, causing ω6 essential fatty acids (EFA) deficiency, which is responsible for the development of a wide variety of diseases, such as atopic eczema, diabetic neuropathy, rheumatoid arthritis, and cardiovascular, reproductive and autoimmune disorders. The therapeutic effects of GLA on cancer, viral infections, osteoporosis and alcoholism have also been proved (Gunstone, 1992; Horrobin, 1992). The fatty acid of SDA is a metabolite of ALA and also acts as a precursor for the long chain omega-3 fatty acids of eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3). SDA in both animal and human is normally synthesized from ALA via the rate-limiting enzyme Δ^6- desaturase (Croda Chemicals Europe Ltd, 2006; Guil-Guerrero et al., 2006a). The use of SDA is increasing due to its applications in several abnormalities and diseases, such as acne and prostate cancer, several cancer types, skin drying, alcoholism, inflammation, thrombosis and rheumatoid arthritis (Guil-Guerrero et al., 2005).

Valuable amounts of GLA have been found in the plant families Asteraceae, Onagraceae, Primulaceae,

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Ranunculaceae, Saxifragaceae, and Scrophulariaceae (Gunstone, 1992), but Boraginaceae is known as the best natural resource of GLA and is the only family including species, which contain more than 20% GLA out of the total seed fatty acids (Erdemoglu et al., 2004). The common plant sources of GLA are evening primrose (*Oenothera biennis* L., 9.6%) (Hudson, 1984), borago (*Borago officinalis* L., 23%) (Guil-Guerrero et al., 2003) and black currant (*Ribes nigrum* L.*,* 15 to 20%) (Lercker et al., 1988), while *Echium* species (Boraginaceae) are the main wild sources for SDA (Guil-Guerrero et al., 2006a). SDA has only been detected together with GLA in seed oils of Boraginaceae, Primulaceae and in plants of the genus *Ribes* (Saxifragaceae) (Gunstone, 1992; Tsevegsuren and Aitzetmuller, 1996; Guil-Guerrero et al., 2006a; Özcan, 2008).

The *Echium* genus contains several endemic frutescent species dispersed across Europe, the Mediterranean region, Macaronesia and North Africa (Guil-Guerrero et al., 2000c, 2001b; Croda Chemicals Europe Ltd, 2006). Several studies have reported on the fatty acid composition of seed oil from different species of Boraginaceae family.

New sources of GLA and SDA have been reported; among these are European and Macaronesian *Echium* seed and leaf oils (Guil-Guerrero, 2000b, c, 2001a, b, 2003; López-Martínez et al., 2004; Guil-Guerrero et al., 2006b). There are new findings on essential fatty acids in species from Boraginaceae family that have been described as endemics from Morocco (Guil-Guerrero et al., 2006a) and Turkey (Erdemoglu et al., 2004; Özcan, 2008). According to their data some species can be evaluated as alternative wild sources of GLA and SDA. For SDA, among all surveyed taxa in Turkey, *Echium* species were in top of the range (Özcan, 2008).

Fish oil is the major source of n-3 long chain (≥C20) polyunsaturated fatty acids (n-3 LC-PUFA) in the human diet, it has been proved that feeding fish with oil from the seeds of some species such as *Echium plantagineum* with high concentrations of SDA would result in fish rich in n-3 PUFA and n-3 LC-PUFA, with the resulting seafood being highly suitable for human consumption (Alhazzaa et al., 2011).

A total of 4 *Echium* taxa at the specific level were reported to be present in Iran (Riedl, 1967). In this study we report the fatty acid composition of the seed, leaf and stem oils of six populations from two shrubby *Echium* species. Until now, these species have not been evaluated with respect to fatty acids content and searching for new sources of physiologically active fatty acids. This is the first study on the fatty acid composition of the oils from *Echium* species growing in Iran.

MATERIALS AND METHODS

Plant materials

Seeds of two *Echium* species were collected at maturity from different regions of Iran in the natural habitats of plants. Seeds of three populations of *E. amoenum* Fisch and C.A. Mey were obtained from three mountainous areas (Behshahr, Hezar Jarib and Jannat Roodbar- Ramsar) in Mazandaran province during August 2009. Leaves and stems were also harvested from wild plants in June 2009.

Seeds of three populations of *E. italicum* L. were gathered from three different areas: Boumehen in Tehran province, Kaleybar in East Azerbaijan province and Alamut in Qazvin province during August to September 2009. Leaves and stems of *E. italicum* plants were harvested from the mentioned areas in June 2009. Table 1 shows the collection sites and times for each kind of plant. The voucher specimens are kept in Tehran University Central Herbarium (TUH).

Oil extraction and transesterification

Plant organs were freeze-dried and ground to a powder in a mortar. About 1 g of each sample was used for oil extraction with a Soxhlet apparatus and n-hexane as the extracting solvent. Free fatty acids (FFAs) were prepared by the saponification of sample oils (López-Martínez et al., 2004). A hydroalcoholic solution was prepared by dissolving 0.48 g of NaOH and 0.005 g Na_2EDTA in 1.6 ml of water and then 1.6 ml of ethanol was added. A mixture containing 1 g of oil and 2 ml of the hydro-alcoholic solution was heated at 60°C with magnetic stirring at 550 rpm for 1 h. Then 0.40 ml of water and 4 ml of hexane were added and the solution was stirred for 1 h. The upper phase containing unsaponificable matter was removed and discarded. Then, the pH of the lower layer was adjusted to 2.0 with drop wise adding of hydro-chloride solution. The obtained FFAs were extracted by the addition of hexane and the hexane layer was separated. The FFA-containing upper layer was dehydrated with anhydrous magnesium sulfate and the solvent was evaporated in a vacuum rotary evaporator at 35°C. FFAs were methylated according to the method of Lepage and Roy (1984). Fatty acid methyl esters were obtained by heating the FFA mixtures in test tubes containing 1 ml of the methylation mixture (methanol/ acetyl chloride, 20: 1 v/v) and 0.5 ml of hexane and heated at 100°C for 10 min. After cooling to room temperature, 1 ml of distilled water was added, and the upper hexane layer was removed for GC analysis. All assays were performed in triplicate and variations among samples were routinely less than 5%. Mean values of different experiments are shown in the Table 2.

Gas chromatography analyses

Mixed fatty acid methyl esters (FAME) were analyzed in a Young Lin ACM 6000 series gas chromatograph (Young Lin Instrument Co., Ltd, Korea) equipped with an ionization flame detector (FID). The separation was carried out in a TR-CN100 poly (biscyanopropyl) siloxane capillary column (60 m × 0.25 mm i.d. × 0.20 µm film thickness; Teknokroma, Barcelona, Spain). The flow of

Table 1. The collected plant materials, collection sites and dates of collection. TUH = Tehran University Central Herbarium.

Species	Locality	Climate	Latitude	Longitude	Altitude [m]	Date	TUH no.
Echium italicum L.	Tehran province-Boumehen	Cold and semi-humid	35º 44´	51º 52´	1500 to 2000	June and September 2009	38891
Echium italicum L.	East Azerbaijan province-Kaleybar	Temperate and semi-humid	38º 52´	47º 02´	1500 to 2000	June and August 2009	38892
Echium italicum L.	Qazvin province-Alamut	Cold and semi-humid	36º 27´	50º 23´	1000 to 1500	June and September 2009	38893
Echium amoenum Fisch & C.A. Mey	Mazandaran province-Hezar Jarib	Cold and semi-humid	36º 40´	53º 20´	2500 to 3000	June and August 2009	38894
Echium amoenum Fisch & C.A. Mey	Mazandaran province-Behshahr	Temperate and humid	36º 41´	53º 32´	50 to 200	June and August 2009	38895
Echium amoenum Fisch & C.A. Mey	Mazandaran province-Ramsar	Temperate and humid	36º 53´	49º 40´	1500 to 2000	June and August 2009	38896

Table 2. Oil content and fatty acid composition in different organs of six populations from two Iranian *Echium* species. Data are expressed as mean ± SD. Mean values within the same column sharing a common letter are not significantly different (P <0.05).

Species (population)	Organ	Oil% (wt)	GLA (%)[a]	Saponifiable fatty acids[b]							
				GLA	16:0	18:0	18: 1ω9	18:2ω6	18:3ω3	18:4ω3	GLA/SDA[c]
E. italicum (Boumehen)	Seed	28.40	2.19	7.70±0.18^{c}	6.51±0.15^{m}	3.67±0.09^{j}	16.10±0.15^{b}	14.09±0.29^{o}	36.61±1.58^{d}	10.47±0.85^{b}	0.74
E. italicum (Kaleybar)		6.20	0.61	9.79±0.28^{a}	11.52±0.28^{j}	3.67±0.07^{j}	16.23±0.19^{a}	15.61±0.36^{m}	29.95±0.43^{i}	12.45±1.05^{a}	0.77
E. italicum (Alamut)		19.40	0.76	3.94±0.15^{fg}	18.93±0.35^{g}	4.30±0.07^{f}	12.63±0.14^{g}	20.15±0.37^{h}	22.12±0.29^{l}	4.33±0.16^{i}	0.91
E. amoenum (Hezar Jarib)		22.00	0.89	4.05±0.16^{f}	27.40±0.74^{b}	4.19±0.12^{fg}	13.17±0.28^{e}	21.81±0.24^{f}	44.15±0.89^{a}	5.02±0.56^{f}	0.81
E. amoenum (Behshahr)		22.40	1.30	5.73±0.19^{e}	7.58±0.18^{k}	4.89±0.04^{c}	15.61±0.25^{c}	26.94±0.18^{a}	33.51±0.91^{h}	4.80±0.83^{g}	1.19
E. amoenum (Ramsar)		33.80	2.07	6.13±0.20^{d}	7.02±0.16^{l}	3.83±0.07^{i}	14.96±0.019^{d}	18.26±0.59^{j}	39.95±0.86^{c}	8.83±0.18^{c}	0.69
E. italicum (Boumehen)	Leaf	2.33	0.09	3.93±0.05^{fg}	23.83±0.29^{d}	5.32±0.11^{b}	8.78±0.09^{n}	11.22±0.12^{q}	35.43±0.28^{f}	2.61±0.03^{m}	1.51
E. italicum (Alamut)		2.44	0.09	3.49±0.12^{h}	16.10±0.19^{h}	4.08±0.017^{h}	10.69±0.17^{j}	14.13±0.19^{n}	35.54±0.81^{e}	5.29±0.17^{e}	0.66
E. amoenum (Hezar Jarib)		1.60	0.14	8.50±0.14^{b}	23.49±0.31^{e}	6.36±0.18^{a}	8.93±0.21^{m}	11.75±0.24^{p}	24.65±0.96^{k}	1.13±0.05^{p}	7.52
E. amoenum (Behshahr)		5.20	0.20	3.79±0.08^{g}	25.82±0.42^{c}	4.48±0.16^{e}	8.45±0.18^{o}	16.81±0.26^{l}	35.02±0.37^{g}	3.59±0.08^{k}	1.06
E. amoenum (Ramsar)		1.90	0.06	3.04±0.09^{i}	11.97±0.13^{i}	2.42±0.02^{k}	11.06±0.06^{i}	17.19±0.18^{k}	42.90±0.87^{b}	7.89±0.15^{d}	0.43
E. italicum (Boumehen)	Stem	1.43	0.04	2.47±0.04^{j}	23.52±0.30^{de}	4.55±0.08^{e}	8.92±0.03^{m}	21.85±0.38^{e}	24.65±0.39^{k}	2.56±0.08^{o}	0.96
E. italicum (Kaleybar)		1.00	0.03	2.87±0.09^{i}	20.42±0.27^{f}	3.62±0.04^{j}	12.94±0.18^{f}	18.81±0.49^{i}	26.35±0.52^{j}	4.54±0.16^{h}	0.63
E. italicum (Alamut)		0.92	0.04	4.19±0.14^{f}	23.83±0.36^{d}	4.75±0.09^{d}	12.57±0.19^{h}	20.63±0.41^{g}	19.55±0.49^{o}	3.02±0.07^{l}	1.39
E. amoenum (Hezar Jarib)		0.80	0.03	3.33±0.10^{h}	29.28±0.29^{a}	6.31±0.09^{a}	10.22±0.17^{k}	25.68±0.35^{c}	20.99±0.79^{n}	3.99±0.05^{j}	0.83
E. amoenum (Behshahr)		0.70	0.02	2.28±0.07^{j}	25.76±0.40^{c}	6.39±0.10^{a}	9.99±0.05^{l}	24.22±0.37^{d}	15.45±0.43^{p}	0.98±0.02^{q}	2.33
E. amoenum (Ramsar)		0.84	0.03	4.05±0.09^{f}	23.56±0.35^{e}	4.16±0.06^{gh}	8.15±0.03^{p}	26.34±0.23^{b}	21.06±1.01^{m}	2.58±0.14^{n}	1.57

[a]GLA percentage over the organ dry weight, and are obtained as the mean value of three replicate experiments[b]. Fatty acid percentage over the total organ oil. [c] γ-Linolenic acid: stearidonic acid ratios.

the carrier gas (He) was 1 ml/min. Injector and detector temperature was 290°C. Split ratio in the injector was 25:1. The oven temperature profile was as follows: 175°C (2 min), 3°C per min to 230°C, 230°C (3 min), giving a total heating time of about 25 min. Injection volume was 1 µl and a blank was run after every ten analyses. Peaks were identified by comparison with known methyl ester standards (C16 to C20 saturated and unsaturated fatty acids, from Sigma). Heptadecanoic acid (17:0) methyl ester was used as internal standard for quantitative analyses.

Gas chromatographic-mass spectrometry analysis

Verification of double bonds and hydrocarbons was achieved by GC-mass spectrometry (MS) in an Agilent HP 6890N G.C. provided with an

Agilent HP 5973 M.S. A TR-CN100 poly (biscyanopropyl) siloxane capillary column (60 m × 0.25 mm i.d. × 0.20 µm film thickness; Teknokroma, Barcelona, Spain) was used. The flow of the carrier gas (He) was 1 ml/min. Injector temperature was 290°C, and the pressure at the head of the column was 5 psi. The oven starting temperature was 175°C (2 min) and it was increased at a rate of 3°C/min until 230°C (3 min). The temperature in the inter-phase was 290°C and the temperature of the source in the detector was 220°C.

Statistical analysis of data

Data obtained from three independent analyses of samples were expressed as means (± SD). Statistical differences between means were calculated by Duncan's test for multiple comparisons (SPSS 15.0). A p-value <0.05 was considered statistically significant.

RESULTS

Oil content and fatty acid composition of different organs from six populations of two *Echium* species analyzed here are given in Table 2. Seed oil content ranged from 6.2% in *E. italicum* (Kaleybar population) to 33.8% in *E. amoenum* (Ramsar population). The GLA content on total seed oil weight reached a maximum of 2.19% for *E. italicum* (Boumehen population). The percentages of GLA on total fatty acids ranged from 3.94 to 9.79% in *E. italicum*, populations of Alamut and Kaleybar, respectively. The SDA content related to total fatty acids was also significant in seed oils, mainly in *E. italicum* plants, with values varying from 4.33 (Alamut population) to 12.45% (Kaleybar population).

The main unsaturated fatty acid in seed oils was ALA, ranging from 22.12% in *E. italicum* (Alamut population) to 44.15% in *E. amoenum* (Hezar Jarib population). LA was also present in all seed oil samples, with values varying from 14.09% in *E. italicum*, (Boumehen population) to 26.94% in *E. amoenum* (Behshahr population). With respect to the amount of the main monounsaturated fatty acid, oleic acid (OA, 18:1ω9), we obtained similar values for all seed oil samples. Oleic acid (OA) ranged from 12.63 to 16.23% in *E. italicum*, populations of Alamut and Kaleybar, respectively.

The saponificable oil content in leaves of the analyzed *Echium* plants varied from 1.6 to 5.2% in Hezar Jarib and Behshahr populations of *E. amoenum*, respectively. The percentages of total leaf oil weight varied from 0.06% GLA in *E. amoenum* from population of Ramsar to 0.2% GLA in *E. amoenum* from Behshahr population. The content of GLA ranged from 3.04 to 8.5% of total fatty acids in leaf oils of *E. amoenum* plants from Ramsar and Hezar Jarib populations, respectively.

Leaf oil of *E. amoenum* (Ramsar population) constituted the richest source of SDA (7.89%) in all analyzed samples. The main unsaturated fatty acid component of leaf oils was ALA, ranging from 24.65 to 42.90% of total fatty acids in *E. amoenum* populations. LA content ranged from 11.22% of total fatty acids in *E. italicum* (Boumehen population) to 17.19% in *E. amoenum* (Ramsar population). OA was measured in the highest amount (11.06%) in leaf oil of *E. amoenum* (Ramsar population).

Stem oil content varied from 0.7% in *E. amoenum* (Behshahr population) to 1.43% in *E. italicum* (Boumehen population). The GLA value ranged from 0.02% of total stem oil weight in *E. amoenum* (Behshahr population) to 0.04% in *E. italicum* (Alamut population). The percentages of GLA ranged between 2.28% of total fatty acids in stem oil of *E. amoenum* (Behshahr population) to 4.19% in *E. italicum* (Alamut population). The amount of SDA in stem oil of *Echium* plants was determined relatively low, with the lowest content (0.98%) given in *E. amoenum* (Behshahr population) and the highest content (4.54%) given in *E. italicum* (Kaleybar population). Among the studied plants, stem oil of *E. amoenum* from Hezar Jarib population had the lowest ALA content (15.45%), while *E. italicum* from Kaleybar population had the highest value (26.35%). The amount of LA in the stem oils ranged from 18.81% in *E. italicum* from Kaleybar population to 26.34% in *E. amoenum* from Ramsar population. The OA content of stem oils was within the range of 8.15% in *E. amoenum* (Ramsar population) to 12.94% in *E. italicum* (Kaleybar population).

The range of variations in the total ratios of fatty acid groups in seed oils were in the order poly-unsaturated (50.54 to 75.03%), mono-unsaturated (12.63 to 16.23%), and saturated fatty acids (10.18 to 31.59%) (Table 3). Total percentage of unsaturated fatty acids was high (63.17 to 88.20%). Data regarding leaf and stem oils are also shown in Table 3. Significant differences were found for mono and poly-unsaturated fatty acids among organs at inter and intra-species levels (Table 2) ($P < 0.05$). The ratios of linoleic to α-linolenic acid showed considerable variations, especially in seed and stem oils of different populations of both species. In general, all organs of studied plants here have substantial contents of linoleic and linolenic acids for food intakes (FNB, 2005).

Seed oil of *E. italicum* (Boumehen and Kaleybar populations) and leaf oil of *E. amoenum* (Hezar Jarib and Ramsar populations) for GLA and SDA exhibited the highest concentrations. Seed and leaf oils of *E. amoenum* (Hezar Jarib and Ramsar populations) were also rich sources for α-ALA. Higher levels of LA occurred in the seed and stem oils of *E. amoenum* plants.

DISCUSSION

Compared to other studied, *Echium* species

Table 3. Total oils, total amounts of fatty acids and some ratios of fatty acids (%) in different organs of examined *Echium* plants.

Species (population)	Organ	Total oil	Saturated	Mono-unsaturated	Poly-unsaturated	Unsaturated in total	Linoleic/ α- Linolenic	α/γ- Linolenic	ω3/ω6	Unsaturated/ saturated	Poly/ monounsaturated
E. italicum (Boumehen)	Seed	28.40	10.18	16.10	68.87	84.97	0.38	4.75	2.16	8.35	4.28
E. italicum (Kaleybar)		6.20	15.19	16.23	67.80	84.03	0.52	3.06	1.67	5.53	4.18
E. italicum (Alamut)		19.40	23.23	12.63	50.54	63.17	0.91	5.61	1.10	2.72	4.00
E. amoenum (Hezar Jarib)		22.00	31.59	13.17	75.03	88.20	0.49	10.90	1.90	2.79	5.70
E. amoenum (Behshahr)		22.40	12.47	15.61	70.62	86.23	0.80	5.85	1.17	6.91	4.52
E. amoenum (Ramsar)		33.80	10.85	14.96	73.17	88.13	0.46	6.52	2.00	8.12	4.89
E. italicum (Boumehen)	Leaf	2.33	29.15	8.78	53.19	61.97	0.32	9.02	2.51	2.13	6.06
E. italicum (Alamut)		2.44	20.18	10.69	58.45	69.14	0.40	10.18	2.31	3.43	5.48
E. amoenum (Hezar Jarib)		1.60	29.85	8.93	46.03	54.96	0.48	2.90	1.27	1.84	5.15
E. amoenum (Behshahr)		5.20	30.30	8.45	59.21	67.66	0.48	9.24	1.87	2.23	7.00
E. amoenum (Ramsar)		1.90	14.39	11.06	71.02	82.08	0.40	14.11	2.51	5.70	6.42
E. italicum (Boumehen)	Stem	1.43	28.07	8.92	51.53	60.45	0.89	9.98	1.12	2.15	5.78
E. italicum (Kaleybar)		1.00	24.04	12.94	51.93	64.87	0.71	9.18	1.42	2.70	4.01
E. italicum (Alamut)		0.92	28.58	12.57	47.39	59.96	1.05	4.67	0.90	2.10	3.77
E. amoenum (Hezar Jarib)		0.80	35.59	10.22	53.99	46.21	1.22	6.30	0.86	1.80	5.28
E. amoenum (Behshahr)		0.70	32.15	9.99	42.93	52.92	1.57	6.78	0.62	1.65	4.30
E. amoenum (Ramsar)		0.84	27.72	8.15	54.03	62.18	1.25	5.20	0.78	2.24	6.63

(Guil-Guerrero et al., 2000b, 2001a, b; Erdemoglu et al., 2004; López-Martínez et al., 2004, 2005; Guil-Guerrero et al., 2006a, b, 2007, 2008; Özcan 2008), GLA values for seed oils of Iranian *Echium* plants exhibit moderate per-centages with the highest one (9.79%) being for *E. italicum* (Kaleybar population). There were significant differences (Duncan's test, P <0.05) in GLA content and also fatty acid profiles between different populations of *Echium* species as well as different organs (Table 2). The conspicuous presence of higher levels of GLA in the leaves (8.5% in *E. amoenum,* Hezar Jarib population) and stems (4.19% in *E. italicum,* Alamut population) of Iranian *Echium* species to those in other studied *Echium* plants (Guil-Guerrero et al., 2000b, 2001a), can be considered as a good advantage for these species. GLA was found in Macaronesian *Echium* leaves, ranging from 0.86% in *E. wildpretii* to 4.94% in *E. bethencourtianum*. Low amounts of GLA were also found in the leaf oils of European *Echium* plants, ranging from 0.26% in *E. boissieri* to 1.94% in *E. creticum*. *Borago offcinalis* had the highest GLA content (4.76%) (Guil-Guerrero et al., 2000b). GLA was detected mainly in seed and root tissues of forty-nine plant species from Spain belonging to the Boraginaceae, Scrophulariaceae, Onagraceae, and Ranunculaceae families (Guil-Guerrero et al., 2001a). According to this data in addition to seeds, stems and leaves of *Echium* plants can be used as other natural sources of GLA.

Among the Iranian *Echium* plants examined for seed oil in this report, four populations showed SDA values higher than 5% and two showed SDA percentages in total seed oil of about 3%. *E. italicum* (Kaleybar population) seed oil was the richest source of SDA, with 12.45% on the total fatty acids. Compared with previous reports for other plants of the *Echium* genus and some species belonging to genera from the Boraginaceae family rich in polyunsaturated fatty acids (Guil-Guerrero et al., 2000c, 2001a, b; López-Martínez et al., 2004, 2005; Guil-Guerrero et al., 2006a, b, 2008; Özcan, 2008), some Iranian species of *Echium* appear to be potential new sources of SDA which also possess noticeable

amounts of GLA. Although, SDA was reported in relatively high amounts, mainly in some European *Echium*, with the highest values of 14.24% in *E. creticum* leaf oil (Guil-Guerrero et al., 2000b), 14.59% in *E. italicum* seed oil (Özcan, 2008) and also 16.2% in seed oil of *E. humile* ssp. *pycnanthum* (Pomel) Greuter and Burdet, a Moroccan species (Guil-Guerrero et al., 2006a). In the top of the range was seed oil of *E. asperrimum*, with SDA content of 21.06% (Guil-Guerrero et al., 2001a). Compared to many Macaronesian and some European studied *Echium* species (Guil-Guerrero et al., 2000b, 2001a), relatively high amounts of SDA were also found in leaf and stem oils of certain Iranian *Echium* plants such as *E. amoenum* (Ramsar population) and *E. italicum* (Kaleybar and Alamut populations).

Conversely, the presence of higher ratio levels of ALA to GLA, especially in leaves and stems was a markedly characteristics of all *Echium* species considered here. Since the SDA is a metabolite of ALA (Guil-Guerrero et al., 2006a), our results indicated a positive correlation between the amounts of SDA and ALA in oil samples. While oils with higher amounts of ALA showed higher values of SDA. A survey on fatty acid composition in leaf oil of Macaronesian *Echium* plants revealed that the main unsaturated fatty acid was ALA, ranging from 9.32% in *E. acanthocarpum* to 54.45% in *E. simplex* (Guil-Guerrero et al., 2000b), although the highest values for ALA in Iranian *Echium* plants were obtained for seed (44.15%) and leaf (42.90%) oils of Hezar Jarib and Ramsar populations of *E. amoenum*, respectively. ALA was the most abundant fatty acid in all organs of Iranian *Echium* plants. It seems that our findings are in agreement with previous reports (Guil-Guerrero et al., 2000c, 2001b) about differences in GLA and ALA contents of seeds in some *Echium* species due to the particular environmental conditions, as well as their biological cycle. It is known that fatty acid composition in lipids determines their fluidity and high contents of GLA in seed storage lipids might negatively affect the mobilization of triglycerides during seed germination of plants, especially in areas with lower average temperatures. It has been reported that environmental factors can affect GLA and other poly-unsaturated fatty acids content in the seed oil of Boraginaceae plants (Guil-Guerrero et al., 2001b). In our experience environmental factors seem to affect the fatty acid profiles in *Echium* plants significantly. For instance, considerably different results were obtained for GLA, SDA and ALA profiles (more than 1% variation at least) of seed as well as leaf and stem oils in some populations of *E. italicum*, the same can be concluded for *E. amoenum* plants. For studied plants, the high relative values of SDA, GLA and ALA in oils were not paralleled by high oil content within the organs. The highest GLA value (9.79%) in seed oil was obtained for *E. italicum* (Kaleybar population) with lowest oil over dry weight (6.20%). The same results were true for leaf and stem oils. The common characteristic of all oil samples was the presence of relatively high amounts of LA. LA is converted to GLA by the enzyme Δ^6-desaturase (Guil-Guerrero et al., 2007). A Δ^6-desaturase gene was isolated from *E. plantagineum* and its catalytic activity confirmed by heterologous expression in tobacco plants and yeast. The results have demonstrated that this enzyme is able to carry out Δ^6-desaturation of the ω3 and ω6 fatty acids substrates LA and ALA with an apparent preference for LA (Zhou et al., 2006). On the other hand, expression of the coding sequences of Δ^6-desaturases genes from two species of *E. sabulicola*, and *E. gentianoides* in yeast demonstrated a similar utilization of both LA and ALA for the desaturase of *E. sabulicola*, while a preference for LA over ALA was observed for the enzyme of *E. gentianoides*. Comparison among the amino acid sequences of these desaturases and other related enzymes, showed the possible involvement of some specific positions in the determination of substrate specificity (García-Maroto et al., 2006). Thus lower proportions (below or near the unit) of GLA/SDA (Table 2) and the elevated ω3: ω6 ratios (Table 3) indicated that ALA availability was higher in Iranian *Echium* plants and therefore resulting in an increased SDA contents, especially in seeds. This is likely due to different substrate (LA/ALA) availability and a different activity of ω3-desaturase that regulates metabolite flux towards ω3 fatty acids during seed development. However, in spite of lower ratios of LA/ALA, higher GLA/SDA proportions were detected in leaf oils of some samples. One explanation for the observed bias in the GLA/SDA leaf oil ratios is the existence of Δ^6-desaturases enzymes with different specificities in different organs that determine the utilization rate of two substrates for the synthesis of relevant products (Garcیa-Maroto et al., 2006).

Guil-Guerrero et al. (2000c, 2001a) have performed a multivariable data analysis based on the fatty acid composition in order to investigate relationships among some Macaronesian *Echium* species. They separated some species from the others due to the high content of LA in their seed oils. Moreover, they explained that high contents of LA perhaps can be due to adaptation of those species to their habitats or to their biennial cycle. Consistent with previous reports on seed oils composition of some *Echium* species and other Boraginaceous plants, our results showed that higher amounts of LA in oil samples were accompanied with lower contents of GLA (Guil-Guerrero et al., 2006a, 2008). Iranian *Echium* species are annual herbaceous plants that are commonly grown in highlands with semi-humid to humid and temperate to cold climates. Seed yield potential and oil quality of different wild populations of *E. plantagineum* have been evaluated in different environments and seeding dates, with emphasis on GLA and SDA contents by Berti et al. (2007). No significant differences were observed for GLA and SDA contents of studied populations in different environments. Based on their results, genetic diversity identified among the wild

populations, although not very high, was a valuable source for development of improved commercial cultivars with better agronomic characteristics, higher seed yield, seed oil content and GLA and SDA contents. Whether the fact that high concentrations of LA in oil samples of all *Echium* species tested in this research might confer an adaptive advantage to their environments in these plants, needs further investigation.

In addition, data for total ratios of saturated, mono-unsaturated, and poly-unsaturated fatty acids and considerable differences of some fatty acid proportions (Table 3), showed the amount of unsaturated fatty acids, including poly-unsaturated fatty acids is very high in samples, which is an important issue. Also, in addition to fatty acids such as GLA, SDA and ALA, the amount of LA was high. Since all the fatty acids mentioned are omega-3 and omega-6 fatty acids, they are very important in pharmaceutical and cosmetic industries. It has been mentioned in some reports that leaves, stems and flowers of *Echium* plants are used as food. Young leaves and shoots contain similar properties to borage and can be treated and eaten like spinach in salads (Launert, 1981; Chiej, 1984; Phillips and Fox, 1990; Bremness, 1994). The dried herb, young leaves and flowering tops are also used for medicinal purposes (Chiej, 1984; Foster and Duke, 1990; Chevallier, 1996). *Echium italicum* plants are cultivated in some northern regions of Iran for food and medicinal uses.

According to our results, oils from different organs, especially seed of *E. italicum* showed GLA and SDA amounts noticeably higher than those of *E. amoenum* plants. This fact indicates that fatty acid profiles of the oils can differ between related species or among populations of a species and probably reveal their minor genetic variations and distant taxonomic relationship. This is in good concurrence with some reports (Guil-Guerrero et al., 2000b, 2001b, 2003) about the differences in GLA levels found in continental and Macaronesian *Echium* species. However, this assumption requires further studies.

Among the *Echium* plants studied in the present work, two populations of *E. italicum* (Boumehen and Kaleybar) with valuable contents of GLA and SDA and a population of *E. amoenum* (Ramsar) with the highest total lipid content (33.8%) and relatively considerable GLA, SDA and ALA values of matured seeds and leaves, appear to be potential new sources of ω3 and ω6 fatty acids. In addition, our findings can increase the interest in the cultivation of these plants as annual producer species of GLA or SDA, due to their short biological cycle.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Research Council of Shahed University for financial support during the course of this research. The authors also thank the Institute of Medicinal Plants (ACECR, Karadj, Iran) for GC and GC-MS analyses.

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