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Original article

γ-LINOLENIC ACID CONTENT AND FATTY ACID PROFILES OF THE SEED OILS OF SOME *ANCHUSA* SPECIES

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Abstract

In this study, the fatty acid composition of the seed oils of four Anchusa L. (Boraginaceae) species [Anchusa azurea Miller var. azurea, Anchusa leptophylla Roemer & Schultes subsp. leptophylla, Anchusa arvensis (L.) Bieb. subsp. orientalis (L.) Nordh and Anchusa undulata L. subsp. hybrida (Ten.) Coutinho] collected from different localities in Turkey have been investigated. Fatty acid compositions of the seeds were determined by capillary gas chromatography-mass spectrometry (GC-MS). The seed oil content ranged from 11.20 % in A. azurea var. azurea to 22.85 % in A. leptophylla subsp. leptophylla. The fatty acid composition of the studied Anchusa taxa was uniform. The main fatty acid methyl esters were determined to be oleic (29.0-42.7 %), linoleic (12.5-32.0 %), erucic (3.4-17.9 %), palmitic (4.4-16.3 %) and α-linolenic (0.3-10.8 %) acids. The content of GLA ranged from 3.3 % in A. leptophylla subsp. leptophylla to 6.7 % in A. arvensis subsp. orientalis. As a conclusion, these seed oils may not be considered as new sources of GLA because of low amounts of GLA in the studied Anchusa species.

Key words: *Boraginaceae, Anchusa, γ-Linolenic acid, Fatty acid, Gas chromatography*

Bazı *Anchusa* Türlerinin Tohum Yağlarının γ-Linolenik Asit İçeriği ve Yağ Asiti Profilleri

Bu çalışmada, Türkiye'de farklı lokalitelerden toplanan dört Anchusa L. (Boraginaceae) türünün [Anchusa azurea Miller var. azurea, Anchusa leptophylla Roemer & Schultes subsp. leptophylla, Anchusa arvensis (L.) Bieb. subsp. orientalis (L.) Nordh ve Anchusa undulata L. subsp. hybrida (Ten.) Coutinho] tohum yağlarının yağ asiti bileşimi incelenmiştir. Tohumların yağ asiti bileşimleri kapiller gaz kromatografisi-kütle spektrometrisi (GC-MS) ile tespit edilmiştir. Tohum yağı içeriği A. azurea var. azurea'da % 11.20'den A. leptophylla subsp. leptophylla'da % 22.85'e değişmektedir. Çalışılan Anchusa taksonlarının yağ asiti bileşimi benzer yapıdadır. Başlıca yağ asiti metil esterleri oleik (% 29.0-42.7), linoleik (% 12.5-32.0), α-linolenik (% 0.3-10.8), erusik (% 3.4-17.9) ve palmitik (% 4.4-16.3) asit olarak belirlenmiştir. GLA içeriği A. leptophylla subsp. leptophylla'da % 3.3'den A. arvensis subsp. orientalis'de % 6.7'ye değişmektedir. Sonuç olarak, çalışılan Anchusa türleri düşük miktarlarda GLA içerdiklerinden dolayı, bu tohumların yağları yeni GLA kaynakları olarak değerlendirilmemiştir.

Anahtar kelimeler: *Boraginaceae, Anchusa, γ-Linolenik asit, Yağ asiti, Gaz kromatografi*

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INTRODUCTION

The Boraginaceae Juss. family contains herbs, shrubs and trees which comprises ca 130 genera and 2300 species in the worldwide (1). In Turkey, it is represented by 44 genera 344 species. The genus *Anchusa* L. is represented by 14 species and 19 taxa in Turkey (2). *Anchusa* is one of controversial genus in family Boraginaceae. It has been subjected to the most variable treatments. According to the molecular systematic evidences, *Anchusa* sensu stricto is paraphyletic genus and needs more studies to clarify problems at inter and infra generic level (3).

The presence of γ-linolenic (GLA, C18:3*n*-6), linolenic (LA, C18:2*n*-6), α-linolenic (ALA, C18:3*n*-3), stearidonic (SDA, C18:4*n*-3) and erucic acids (C22:1*n*-9) in the seed oils in Boraginaceae is of chemotaxonomic importance (4-8). γ-Linolenic acid is the first intermediate in the bioconversion of linolenic acid to long-chain polyunsaturated fatty acid arachidonic acid (AA, 20:4*n*-6). GLA has proved its therapeutic value in the treatment of a wide variety of pathologies such as atopic eczema, diabetic neuropathy, rheumatoid arthritis, cancer, viral infections, osteoporosis and alcoholism. Therefore, therapeutic, nutritional and cosmetic importance of GLA is increasing (9).

Up to date, γ-linolenic acid have reported several families principally Boraginaceae, as well as Caryophyllaceae, Scrophulariaceae, Saxifragaceae, Onagraceae, Aceraceae, Ranunculaceae, Primulaceae and Asteliaceae. From other natural sources such as some mosses, marine algae, fungi and microorganisms were also contained different amounts of GLA (10-13). Evening prime rose (*Oenothera biennis* L.) (14), black current (*Ribes nigrum* L.) (15) and borage (*Borago officinalis* L.) (16) are traditionally used as plant sources of GLA. Therefore, many investigations have recorded that the family Boraginaceae has importance in respect of GLA content (17-24).

The purpose of this study is to reveal amount of GLA and the fatty acid profiles of the seed oils of four *Anchusa* species [*Anchusa azurea* Miller var. *azurea*, *Anchusa leptophylla* Roemer & Schultes subsp. *leptophylla*, *Anchusa arvensis* (L.) Bieb. subsp. *orientalis* (L.) Nordh and *Anchusa undulata* L. subsp. *hybrida* (Ten.) Coutinho], belonging tribe Boragineae (Boraginaceae) collected from Turkey.

MATERIAL AND METHODS

Plant materials

Plant materials (Table 1) were collected in fruiting periods at maturity from their natural habitats in Turkey. Authenticated voucher specimens were deposited in the Herbarium of HUB.

Oil extraction and transesterification

The seeds were separated from plant materials and dried under shade. The weighed seeds were ground with anhydrous sodium sulfate and then extracted for 4 h with petroleum ether (bp 40-60 °C, Merck Co., USA) in a Soxhlet apparatus. The solvents were evaporated to dryness under vacuum at 40 °C and the seed oils were stored at 4 °C.

In order to analyze the fatty acid composition of the seed oils, the total fatty acids were converted to fatty acid methyl esters (FAMEs) according to Morrison and Smith (25). The seed oils were saponified with 0.5 N methanolic NaOH solution by heating on a steam bath, and then boiled for 2 minutes. Subsequently, 2 mL of boron trifluoride-methanol complex (20 %, Merck Co., USA) was added and the solutions were heated for 2 minutes in a boiling water bath. After cooling to room temperature, each solution was made up to 10 mL by adding saturated NaCl

solution to stop the reaction. The mixture was left for 30 minutes to gather the oily part on the surface of the solution and then, the upper oily layer was solved with light petroleum and the organic layer was separated using Pasteur pipettes. The FAMEs were dissolved in CH_2Cl_2 prior to each analysis and injected into a GC-MS apparatus. Three samples of each species were used to determine the average FA compositions.

Table 1. Locations of *Anchusa* species.

Species	Locality, date of collection
Anchusa azurea Miller var. *azurea* (ADK 2464, HUB)	B4 Ankara: İncek village, around Atılım University, steppe, c. 1000 m, 23.07.2005.
Anchusa leptophylla Roemer & Schultes subsp. *leptophylla* (ADK 2468, HUB)	B4 Ankara: İncek village, around Atılım University, steppe, c. 1000 m, 23.07.2005.
Anchusa arvensis (L.) Bieb. subsp. *orientalis* (L.) Nordh. (ADK 2722, HUB)	B5 Nevşehir: Nevşehir to Ortaköy, between the vineyards, volcanic area, 1247 m, 36660710 E 4276235 N, 19.5.2006.
Anchusa undulata L. subsp. *hybrida* (Ten.) Coutinho (ADK 2723, HUB)	B5 Nevşehir: Nevşehir to Ortaköy, between the vineyards, volcanic area, 1247 m, 36660710 E 4276235 N, 19.5.2006.

Gas chromatography-mass spectrometry

The FAMEs were analyzed using a Trace 2000 GC series gas chromatograph and a Thermo mass spectrometer. The separation was carried out in a SGE BPx70 column (60 m x 0.25mm, 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 1mL/min. The oven temperature was kept at 100 °C for 5 min, programmed to 240 °C at a rate of 4 °C /min and kept constant at 240 °C for 5 min. The injection and source temperatures were 250 °C and 220 °C, respectively. MS interface temperature was 240 °C. The injection volume was 0.5 µL with a split ratio of 1:30. Injections were done in triplicate. EI/MS were recorded at 70 eV ionization energy. Mass range was applied from *m/z* 50 to 650 amu. Scan time was 0.5 sec. with 0.1 interscan delay. Supelco™ 37 components FAME mixture (Catalog no:47885-U) were used for the comparison of the GC chromatograms. The library search carried out using NIST and Wiley GC-MS library and TÜBİTAK-UME library. The relative percentages of separated compounds were calculated from Total Ion Chromatography by the computerized integrator.

RESULTS AND DISCUSSION

Seed oil content and fatty acid composition of four *Anchusa* taxa collected from Turkey, were analyzed by GC-MS. A chromatogram of *A. azurea* var. *azurea* seed fatty acids is shown in Fig. 1. Results of the present study are given in Table 2.

The total oil yields of the studied taxa ranged from 11.20 % in *A. azurea* var. *azurea* to 22.85 % in *A. leptophylla* subsp. *leptophylla*. Nineteen components were identified in *A. azurea* var. *azurea*, *A. leptophylla* subsp. *leptophylla*, *A. arvensis* subsp. *orientalis* and *A. undulata* subsp. *hybrida*, which represented 93.5, 89.1, 89.4 and 91.2 % of the total oil, respectively. The fatty acid composition of the seed oil of *Anchusa* taxa studied was found to be uniform. The unsaturated fatty acid content of the oils was found to be higher than that of the saturated fatty

acids, which was highest in *A. azurea* var. *azurea* (81.4 %) seed oil. The main fatty acid methyl esters were oleic (29.0-42.7 %), linoleic (12.5-32.0 %), erucic (3.4-17.9 %), palmitic (4.4-16.3 %) and α-linolenic (0.3-10.8 %) acids. The GLA amount of the seed oils were found between 3.3 % and 6.7 % and *A. arvensis* subsp. *orientalis* showed the highest content of GLA in the seed oils. The amount of stearidonic acid in the seed oil was found to be present in trace amounts (<0.1 %) in two *Anchusa* species (*A. leptophylla* subsp. *leptophylla* and *A. arvensis* subsp. *orientalis*) and it was not detected in the seed oil of other two *Anchusa* species (*A. azurea* var. *azurea* and *A. undulata* subsp. *hybrida*) (Table 2). The low amounts of GLA determined in this study that the seed oils of *Anhcusa* taxa studied may not be considered as a source of GLA compared with the above mentioned commercial sources.

Table 2. Oil content and fatty acid composition of *Anchusa* species.

Fatty acid	***Anchusa azurea* var. *azurea***	***Anchusa leptophylla* subsp. *leptophylla***	***Anchusa arvensis* subsp. *orientalis***	***Anchusa undulata* subsp. *hybrida***
Caprilic acid (8:0)	0.6	0.6	1.1	0.5
Miristic acid (14:0)	0.1	0.5	0.1	0.1
Cis-10-Pentadecenoic acid (15:1)	nd	nd	nd	0.1
Palmitic acid (16:0)	**8.0**	**4.4**	**9.2**	**16.3**
Palmitoleic acid (16:1*n*-7)	0.1	0.1	0.1	0.3
Margaric acid (17:0)	0.1	0.1	0.3	0.2
Cis-10-Heptadecenoic acid (17:1)	nd	0.1	0.2	0.1
Stearic acid (18:0)	2.7	2.3	2.0	6.3
Oleic acid (18:1*n*-9)	**31.0**	**33.8**	**29.0**	**42.7**
Cis-11-Octadecenoic acid (18:1*n*-7)	nd	0.1	1.0	1.4
Linoleic acid (18:2*n*-6)	**32.0**	**16.5**	**23.8**	**12.5**
γ-Linolenic acid (18:3*n*-6)	**6.2**	**3.3**	**6.7**	**1.1**
α-Linolenic acid (18:3*n*-3)	**0.3**	**4.1**	**10.8**	**1.2**
Gondoic acid (20:1*n*-9)	0.3	0.6	0.3	0.7
Stearidonic acid (18:4*n*-3)	nd	tr	tr	nd
Heneicosanoic acid (21:0)	0.1	0.1	nd	nd
Behenic acid (22:0)	0.5	0.7	0.3	0.5
Erucic acid (22:1*n*-9)	**10.3**	**17.9**	**3.4**	**5.7**
Nervonic acid (24:1*n*-9)	1.2	3.9	1.1	1.5
Oil content (% seed weight)	11.2	22.8	19.8	21.5
Σ Saturated	12.1	8.7	13	23.9
Σ Unsaturated	81.4	80.4	76.4	67.3
Saturated/Unsaturated	0.15	0.11	0.17	0.35
Total	93.5	89.1	89.4	91.2

*GC-MS analyses were replicated three times (Mean RSD value is % 0.1)
nd: not detected; tr: trace, <0.1 %.

Anchusa species reported in the literature (Table 3) have shown the presence of erucic acid in different amounts between 1.3-6.5 % (7,8,19-23,26,27). In our study, erucic acid ranged from 3.4 % in *A. arvensis* subsp. *orientalis* to 17.9 % in *A. leptophylla* subsp. *leptophylla.* Our results were observed to be higher than those reported in the literature.

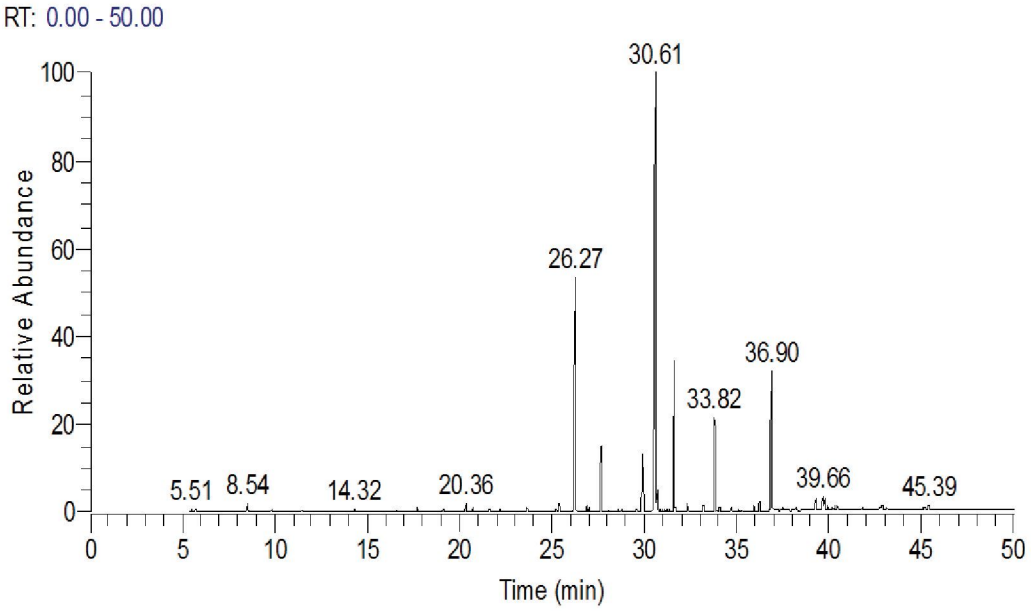

Figure 1. GC-MS spectrum of *Anchusa azurea* var. *azurea* seed oil.

The chemotaxonomic significance of fatty acid composition within *Anchusa* species was emphasized in many studies (7,8,19-23,26,27). According to some authors, the fatty acid composition is important at the tribal level in Boraginaceae family (4,7,8,20). The studies reported in the literature about the seed oil contents and fatty acid compositions of *Anchusa* genus up to now are given in Table 3. *Anchusa azurea* specimens listed in Table 3 were sampled from three continents; Turkey (Asia), Morocco (Africa), Spain and Germany (Europea). The GLA contents of *A. azurea* ranged from 8.2 to 11.11 % in Europe, 11.9 and 13.07 % in Turkey and 8.0 % in Morocco. It seems GLA contents do not show a significant geographical difference. Other fatty acids compositions of *A. azurea* from different regions are also similar. In addition, the GLA values of *A. undulata* ranged from 8.35 to 13.1 %. The samples collected from Turkey, Morocco and Spain did not show a significant difference as regards to GLA content. The GLA amounts are between 3.1-14.15 % for *A. leptophylla* and between 12.9-16.1 % for *A. officinalis*. According to these results, there exists no correlation between species and fatty acid patterns. Our findings were in good agreement with Guil-Guerrero et al.'s results (20). They reported that the seed fatty acid profiles can differ strongly between related species and/or among species populations, reflecting minor genetic variations.

The present study confirmed that the fatty acid profiles of the seed oils in family Boraginaceae were effected by various factors principally related species or populations mentioned above, as well as the minor factors reported in Guil-Guerrero et al.'s study (28) such as degree of ripeness, climate and duration of sunshine. Further studies should be carried out to find new species contain high amount of GLA. The species of the Boraginaceae growing in Turkey, which is represented by 44 genera 344 species should be subjected to much comprehensive studies regarding to fatty acid composition.

Table 3. Literature review of oil content and fatty acid composition of seed oils obtained from *Anchusa* species.

Tür	Oil %	16:0	18:0	18:1 *n*-9 (OA)	18:2 *n*-6 (LA)	18:3 *n*-6 (GLA)	18:3 *n*-3 (ALA)	18:4 *n*-3 (SDA)	20:0	20:1 *n*-9	22:1 *n*-9	Collected sites	Ref.
A. azurea var. *azurea*	8.6	7.7	2.4	23.1	42.3	**11.9**	nd	nd	nd	3.6	4.5	Turkey	23
A. x thirkeana	5.2	8.9	3.1	22.3	14.8	**0.3**	4.6	tr	nd	2.3	1.3	Turkey	23
A. leptophylla ssp. *leptophylla*	22.0	8.6	2.3	21.3	26.4	**3.1**	15.8	4.2	nd	3.1	2.3	Turkey	23
A. leptophylla ssp. *leptophylla*	23.3	8.37	2.2	28.3	26.0	**11.4**	12.8	2.74	0.31	3.33	2.43	Turkey	22
A. undulata ssp. *hybrida*	21.16	8.35	2.1	27.4	27.5	**11.6**	11.9	2.6	0.27	3.47	2.8	Turkey	22
A. azurea var. *azurea*	13.9	8.12	1.49	25.69	38.97	**13.07**	0.22	0.11	0.2	4.05	5.84	Turkey	22
A. leptophylla subsp. *leptophylla*	19.7	7.45	1.77	21.86	28.13	**14.15**	11.40	3.19	0.22	4.07	3.86	Turkey	8
A. arvensis	25.8	7.9	4.1	20.4	24.3	**15.2**	16.3	4.7	nd	nd	nd	Germany	7
A. azurea	13.0	11.3	7.2	20.8	38.1	**10.2**	2.1	0.5	nd	nd	nd	Germany	7
A. officinalis	19.0	9.3	4.6	19.3	32.4	**12.9**	12.7	2.6	nd	nd	nd	Germany	7
A. azurea	21.5	12.2	2.2	32.6	38.5	**8.0**	0.3	nd	0.3	3.5	nd	Morocco	20
A. undulata	28.8	15.3	2.0	31.0	18.0	**1.0**	9.9	4.2	nd	3.7	2.7	Morocco	20
A. undulata ssp. *atlantica*	26.9	13.8	2.2	30.6	18.0	**10.3**	9.5	3.9	nd	4.6	4.7	Morocco	20
A. azurea	22.52	8.63	2.19	24.1	41.78	**11.11**	0.43	0.08	0.23	3.57	0	Spain	19
A. undulata	29.36	8.76	2.1	24.4	25.3	**8.35**	17.9	3.55	0.24	4.21	0	Spain	19
A. azurea	13.9	6.5	1.3	28.7	42.7	**8.2**	nd	nd	nd	3.3	3.9	Spain	26
A. azurea	*	9.22	2.18	30.65	37.0	**8.68**	0.21	0.0	0.23	4.47	6.37	Spain	21
A. azurea	*	nd	nd	27.1	41.2	**10.6**	nd	nd	nd	4.3	6.5	Spain	27
A. officinalis	*	nd	nd	21.7	29.3	**16.1**	13.5	nd	nd	3.4	3.2	Spain	27
A. undulata	*	nd	nd	23.1	28.3	**13.1**	12.4	nd	nd	3.8	3.5	Spain	27

* Oil content of these species is not reported in the literature.
tr: trace; nd: not detected

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