Fatty Acid Profiles of the Seed Oils in Two Groups of *Anchusa officinalis* L.

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Abstract

Total seed oil and fatty acid compositions of two informal groups of *Anchusa officinalis* L. were studied in order to provide additional information for the delineation of the groups. Major fatty acids were linoleic (C18:2n6), oleic (C18:1n9), α-linolenic (C18:3n3), γ-linolenic (C18:3n6), palmitic (C16:0) and stearidonic acid (C18:4n3) respectively. The lower levels were observed in stearic (C18:0), eicosenoic (C20:1n9) and erucic acids (C22:1n9). Linoleic (24,4 to 25,8%) and oleic acids (23,2 to 25,5%) were detected in the highest levels. α-linolenic (14,5 to 15,1%) and γ-linolenic acid (12,5 to 12,6%) as an unusual fatty acid in plants were also at the high concentrations in both groups. Saturated fatty acids in total were observed at the lower levels generally (1,9-8,9%). Significantly difference for the fatty acid profiles between the groups were found ($p<0,05$). Differences were also significant for saturated (palmitic and stearic) and unsaturated fatty acids, and for their some ratios ($p<0,05$). Total percentages of poly-unsaturated (56,27 to 56,41%), mono-unsaturated (26,47 to 31,38%) and saturated fatty acids (10,12 to 11,95%) were quantified closely in both groups. The parameters examined here may be useful as additional biochemical marker set for the discrimination of *Anchusa* at infraspesific levels. Valuable concentrations for essential poly-unsaturated fatty acids (PUFAs) including linoleic, α-linolenic and γ-linolenic acid as special dietetics and nutraceuticals suggest the alternative source potential of *A. officinalis*.

Keywords: *Anchusa officinalis*, taxonomy, seed, fatty acid.

Introduction

Boraginaceae is a very large family covering about 100 genera and 2000 species in the tropical and temperate regions of the world. This family in Turkey comprises about 32 genus and 315 species distributed in different kinds of habitats (Davis 1978). The ratio of endemism at spesific level is very high (%35). Apart from morphological descriptions in the Flora of Turkey and a few studies, detailed taxonomical investigations on *Boraginaceae* in Turkey are very limited (Akçin et al. 2007). Modern revision using consistent parameters is needed.

Micromorphological surface patterns of the fruits were declared to be valuable key characteristics in its taxonomy and diagnosis (Davis 1978). On the other hand, chemical contents of the seeds have characteristically taxonomic importance. It was reported that distributional profiles of fatty acids stored in the seeds are valuable parameters contributing the solution of taxonomical problems at different levels of taxa (Gibbs 1974; Yaniv et al. 1991; Sayanova et al. 1999; Lamarque et al. 2000; Mayworm and Salatino 2002). Fatty acid patterns in the seeds as biochemical tools were also proved to explain the phylogenetic relations in most studies (Hohn and Meinschein 1976; Aitzetmüller et al. 1999). The seeds of *Boraginaceae* were reported to contain very high level of gamma linolenic acid (GLA) having nutritious and biomedical importance in addition to taxonomical utility (Velasco and Goffman 1999; Guil-Guerrero et al. 2001a; 2003).

A limited number of taxa were studied for the fatty acid contents in this family. Rich diversity of Boraginaceae in Turkey were also not investigated sufficiently opart from a few studies (Bağcı et al. 2004; Erdemoğlu et al. 2004). Relatively larger scanning for this family in Turkey based on seed oil characteristics was carried out on 13 genera including 24 taxa, and the taxonomical utility of the contents and alternative sources potential of taxa for essential fatty acids were reported (Özcan 2008, in press, Plant Systematics and Evolution). 15 *Anchusa* species were recorded in the Flora of Turkey distributing in different habitats of Anatolia. The subgenera recognized with using palynological data accounted for distinct entities, but there is no real justification for maintaining them at generic level (Davis 1978). *Anchusa officinalis* L. is a variable species for some morphological features and covering very large distributional areas in Anatolia. It was delineated three informal groups based on some morphological characteristics. The populations of these groups distribute in different regions of Turkey. But, it is questionable that such a few morphological features could delineate the groups, when considering the phenotypic plasticity. It needs some additional consistent parameters for understanding wheather or not such groupings is necessary.

In this study, it was aimed to delineate two groups of *A. officinalis* using fatty acid profiles as additional biochemical markers and shed light on alternative potential of this species for unsaturated fatty acids.

Material and Methods

The plant specimens of two groups of *Anchusa officinalis* L. were collected from its natural localities in Marmara region of Turkey in flowering and seed bearing periods. Plants were determined by the classification criteria in Flora of Turkey (Vol.6) and by comparision with identified specimens from the ISTF (Herbarium of Faculty of Science of Istanbul University).

Mature seed samples of 8-12 gr were collected from five individual specimens in each groups of *A. officinalis*. Air-dried, mature and cleaned seed samples removed impurities for each specimens were ground into meal. Total oil contents were detected in Tecator Soxtec System HT. 3 gr of powdered material from each samples was added to oil cartridge (W1) and 25-50 ml ether into weighted extraction pots (W2) was used. Extraction was carried out (15 min) with rinsing for 30-45 min. The pots were cooled in a dessicator and weighed (W3). The following equation was used to calculate percentages of the oil; Oil % = ((W3-W2)/W1)*100. The oil was transferred into glass sealed amber dark bottles, capped and stored at −18 °C until analysed. IUPAC standart method (Fifth Edition, II.D.19) for the preparation of the fatty acid methyl esters were used. The methyl esters of fatty acids were quantified by Thermoquest Trace GC equipped with a SP-2330 fused silica capillary column (30m, 0.25 mm i.d., 0,20 µm film thickness). The oven temperature was held at 120°C for 2 min and increased at a rate of 5°C/min and held at 220°C for 8 min. Injector and detector temperature were 240°C and 250°C, respectively. Hydrogen was used as carrier gas at a flow rate of 3.5 ml/min. Split flow, split ratio and sample injection was 75 ml/min, 1/150 and 0,5 µl. respectively. Identification and quantification of fatty acid methyl esters was accomplished by comparing the retention times of the peaks with authentic standards (Sigma).

Multivariate analysis of the experimental results were carried out at the $p<0,05$ significance level (SSPS 10.0).

Results

Total seed oil (25,80 to 26,10%) and fatty acid compositions of both informal groups of *A. officinalis* were studied. All analytical results and the ratios of the fatty acids were documented in Table 1. Major fatty acids were linoleic (C18:2n6), oleic (C18:1n9), α-linolenic (C18:3n3), γ-linolenic (C18:3n6), palmitic (C16:0) and stearidonic acid (C18:4n3) respectively. The lower levels were observed in the other fatty acids including stearic (C18:0), eicosenoic (C20:1n9), erucic acids (C22:1n9). The highest quantities were detected for linoleic

(24,4 to 25,8%) and oleic acids (23,2 to 25,5%). α-linolenic acid was also at the high concentrations (14,5 to 15,1%). γ-linolenic acid (C18:3n6) as an unusual fatty acid in plants was determined at considerably high concentrations (12,5 to 12,6%) in both groups.

Saturated fatty acids including palmitic and stearic acid were observed at the lower levels between the groups generally (1,9 to 8,9%). Significantly different values for fatty acid profiles between the groups was observed ($p<0,05$). Saturated, unsaturated fatty acids and some of their calculated ratios were also different significantly ($p<0,05$). Total ratios of poly-unsaturated (56,27 to 56,41%), mono-unsaturated (26,47 to 31,38%) and saturated fatty acids (10,12 to 11,95%) were detected parallelly at different range in both groups.

Total percents of unsaturated fatty acids were between 82,88 to 87,65%. In general, the concentration range of the whole fatty acid profile in Group a of *A. officinalis* was relatively larger than that of Group b. Relatively higher values in Group b for palmitic, stearic, erucic, α-linolenic and stearidonic acid and in Group a for oleic, linoleic and γ-linolenic acid were observed (Fig.1). But, parallel results were obtained for all examined fatty acids in terms of general aspect of the profiles in both groups. The proportions of linoleic to α-linolenic acids, mono-unsaturated and poly-unsaturated fatty acids to saturated fatty acids were relatively higher in Group a. Especially, total ratio of unsaturated fatty acids to saturated ones in Group a showed remarkably higher value. Relatively higher proportions for α-: γ-linolenic acid and poly-: mono-unsaturated fatty acids were examined in Group b. Two groups were segregated based on six different proportions of fatty acids (Fig.2). Valuable concentrations for essential poly-unsaturated fatty acids (PUFAs) including linoleic, α-linolenic, γ-linolenic and stearidonic acid as special dietetics and nutraceuticals were detected in both groups of *A. officinalis* suggesting alternative source potential for these fatty acids used in biomedical, cosmetic and food sectors.

Table 1. The concentrations, total ratios and some proportions of fatty acids between groups.

Taxa	***Anchusa officinalis* (Group a)**	***Anchusa officinalis* (Group b)**
C16:0 Palmitic	8,21	8,90
C18:0 Stearic	1,91	3,05
C18:1n9c Oleic	25,50	23,23
C20:1n9 Eicosenoic	3,24	3,24
C22:1n9 Erucic	2,64	2,66
C18:2n6c Linoleic	25,82	24,48
C18:3n6 γ-Linolenic	12,69	12,59
C18:3n3 α-Linolenic	14,57	15,11
C18:4n3 Stearidonic	3,19	4,23
Total oil (%)	26,10	25,80
Saturated fatty acids	10,12	11,95
Mono-unsaturated fatty acids	31,38	26,47
Poly-unsaturated fatty acids	56,27	56,41
Unsaturated in total	87,65	82,88
α- / γ-linolenic	1,14	1,20
Poly- / Mono- unsaturated	1,79	2,13
linoleic / α-linolenic	1,77	1,62
Mono-unsaturated/Saturated	3,10	2,21
Poly-unsaturated/Saturated	5,56	3,08
Total unsaturated / total saturated	6,12	4,72

Each value for the fatty acid concentrations is the average from dublicate determinations

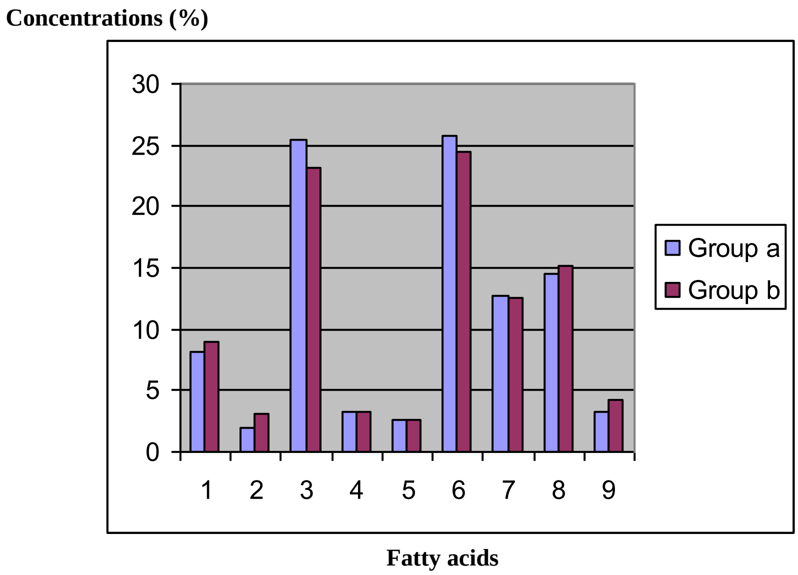

Figure 1. Fatty acid concentrations (%) between groups. 1: C16:0 Palmitic, 2: C18:0 Stearic, 3: C18:1n9 Oleic, 4: C20:1n9 Eicosenoic, 5: C22:1n9 Erucic, 6: C18:2n6 Linoleic, 7: C18:3n6 γ-Linolenic, 8: C18:3n3 α-Linolenic, 9: C18:4n3 Stearidonic acid.

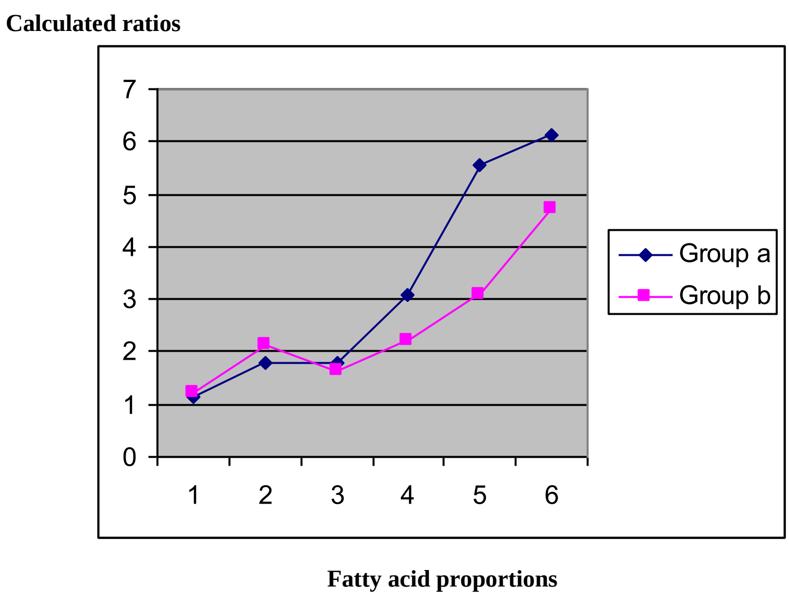

Figure 2. Some proportions of fatty acids between groups 1: α- / γ-linolenic, 2: Poly- / Mono- unsaturated, 3: linoleic / α-linolenic, 4: Mono-unsaturated/Saturated, 5: Poly-unsaturated / Saturated, 6: Total unsaturated / Total saturated.

Discussion

Fatty acid compositions of the seeds at spesific and infraspesific level may account for some taxonomic and phylogenetic relations as additional and consistent parameters. Examined groups here were reported to differ based on its some minor morphological characteristics in the flowers. The features of corolla and calyx are used in dichotomic key for dividing this genera into three informal groups (Chamberlain 1978). It was reported that *A. officinalis* is closely related with *A. leptophylla* and *A. undulata* in terms of morphological characteristics as described in the Flora of Turkey. Fatty acid patterns of these related species were reported to be considerably similar, apart from *A. azurea* that explain distinct morphological and fatty acid characteristics reflecting taxonomical divergency. Relatively higher levels in linoleic and erucic acid, lower levels in α-linolenic and stearidonic acids in *Anchusa azurea* subsp. *azurea* were found compared to other *Anchusa* species (Guil-Guerrero et al. 2001a, b; Erdemoğlu et al. 2004; Özcan 2008, in press, Plant Systematics and Evolution). But, knowing the range of variation in a given group is important for the delimitation of closely related taxa. Our results on oleic, linoleic, α-linolenic, GLA, eicosenoic and erucic acids in the seed oil of *A. officinalis* correspond with the literature (Tetenyi 1974; Velasco and Goffman 1999; Cisowski et al. 2001). On the other side, the proportions of linoleic to α-linolenic acids, mono-unsaturated and poly-unsaturated fatty acids to saturated fatty acids and total ratio of unsaturated fatty acids to saturated ones may provide valuable chemometric data for infraspecific segregation of *A. officinalis* (Fig.2).

Relatively different fatty acid profiles between groups may account for typical characteristics resulting from different enzymatic activitiy encoded by original genotypes. It is needed to know the degree of stability of these parameters between groups. Large scannings may be informative for determining the range of intraspesific variations of *A. officinalis*. However, some proportions of fatty acids examined here may provide more constant and discriminative parameters than its concentrations in delineation of *Anchusa*. But, it would be useful to support our findings with using DNA parameters for identifying the infraspesific ranks of this species and also the genotypes having high product qualities. Fatty acid patterns were declared to reveal lower intraspecific variability and higher taxonomic resolution (Mayworm and Salatino 2002). Characteristic fatty acid profiles at specific level of Boraginaceae were reported from Turkey (Bağcı et al. 2004; Erdemoğlu et al. 2004). Segregation of *Echium*, *Symphytum* and *Anchusa* at generic level were also accomplished with using nine fatty acids examined here (Özcan 2008, in press, Plant Systematics and Evolution). In the broad sense, studied fatty acid data as a marker set may have an utility in the segregation of Boraginaceae at generic and infrageneric levels. Many studies reported phylogenetic relationships parelleled by differences in the fatty profiles of the seed oils (Hohn and Meinschein 1976; Aitzetmüller 1999; Velasco and Goffman 1999). The results on the fatty acid compositions of the seed oils may also contribute some extent the phylogenic relations.

The species having large distributional areas may be more influenced with ecological factors compared to the taxa distributed in limited areas. The range of areals for any species may account for the reason of biochemical polymorphism derived from both orjinal genotypes and the ecological factors. Different maturing stages of the seeds are the other factor in fatty acid compositions of the seed oils (Peiretti et al. 2004). Taxa distributed in relatively limited areals such as endemics may have not remarkable variations for the fatty acid levels. Some variations in seed oil contents and the concentrations of fatty acids were reported from the specimens collected from different localities and climatic conditions of each year, producing decreasing levels of seed oils correlated with dry seasons (Angelini et al. 1997). On the other hand, total seed oil contents in ripened stage between groups examined here were observed at very similar quantities though collected from very different localities, implying possibly its consistent characteristic feature at spesific level. But, relatively lower quantities in total seed oil of *A. officinalis* (21.6%) compared to our study (25,8 to 26,1%) were obtained likely derived from different growing conditions and theripening stages (Cisowski et al. 2001). However, fatty acid compositions and its some proportions may be more valuable and additional data for the discrimination of two groups of *A. officinalis*.

Our results also provide information for the source potential of the studied fatty acids. Considerable high level of mono- and poly-unsaturated fatty acids in addition to total oil contents in *A. officinalis* suggest the utility of this species as alternative industrial crop. Remarkable concentrations of α-linolenic, γ-linolenic and stearidonic acid as unusual fatty acids that are important for biomedical applications were obtained in both groups. This species as an alternative wild resources for unsaturated fatty acids has very large distributional areas in Anatolia. Selected genotypes with high product quality may be cultivated as industrial crops in its natural habitats that is not suitable for conventional agriculture.

Fatty acid profiles of seed oils, total ratios of saturated and unsaturated fatty acids and its relative percentages as an additional chemometric data are seem to be useful for the characterization and delineations at infraspesific levels of *A. officinalis*.

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