

## Analysis Fatty Acids of some *Hyoscyamus*, *Datura*, *Atropa* Species from Azerbaijan

### Azerbaycan'dan Bazı *Datura*, *Hyoscyamus*, *Atropa* Türlerinin Yağ Asitlerinin Analizi

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#### ABSTRACT

**INTRODUCTION:** *Datura stramonium*, *Datura stramonium* var. *tatula*, *Hyoscyamus reticulatus*, *Hyoscyamus niger*, *Atropa caucasica* naturally found in Azerbaijan and their seeds possess 17-35% of oils. Study aim to evaluate and determine fatty acids of these plants special for Azerbaijan climate and geography. The presented study is the first research of fatty acids of *A. caucasica*, which is an endemic species of Caucasus.

**METHODS:** Fatty acids of seed oils were derivatized to methyl esters and analyzed by gas chromatography equipped with a flame ionization detector, in comparison with a standard mixture of 37 fatty acid methyl esters

**RESULTS:** Linoleic (55-79%), oleic (11-26%), palmitic (4-12%) and stearic (2-3%) acids compose 97% of total fatty acids. Other minor compounds, including 2 trans fatty acids, were determined in the samples. Significantly high concentration of a medicinaly important polyunsaturated fatty acid - linoleic acid was observed in all samples.

**DISCUSSION AND CONCLUSION:** The results of this study showed that these oils are especially valuable sources of linoleic and oleic acids, which have beneficial effects on cardiovascular diseases and are important compounds for the pharmaceutical and cosmetic industries in the manufacture of liposomes, nano- and microemulsions, soaps, etc.

**Keywords:** fatty acids, gas chromatography, datura, hyoscyamus, atropa

#### ÖZ

**GİRİŞ ve AMAÇ:** *Datura stramonium*, *Datura stramonium* var. *tatula*, *Hyoscyamus reticulatus*, *Hyoscyamus niger*, *Atropa caucasica* türleri Azerbaycanda doğal olarak bulunmakta ve % 17-35yağ içermektedir. Araştırma bitkilerde olan yağ asitlerinin Azerbaycan iklimi ve coğrafyası için değerlendirilmesi ve tayinini amaçlamaktadır. Sunulan araştırma kafkasyanın endemik türü olan *A.caucasica* –nın yağ asitlerine ait ilk rapordur.

**YÖNTEM ve GEREÇLER:** Tohum yağlarının yağ asitleri metil esterlerine dönüştürülmüş ve

alev iyonlaştırma dedektörü ile birleştirilmiş gaz kromatografisinde, 37 yağ asitinin metil esterlerinin standart nüümnesi ile kıyaslamalı olarak analiz edilmiştir.

BULGULAR: Linoleik (%55-79), oleik (%11-26), palmitik (%4-12) ve stearik (%2-3) asitler toplam yağ asitlerinin %97'sini oluşturmaktadır. Örneklerde 2 trans yağ asidi dahil olmak üzere diğer küçük bileşikler de belirlendi. Tüm numunelerde tıbbi açıdan önemli, çoklu doymamış yağ asidi - linoleik asitin önemli ölçüde yüksek oranı gözlendi.

TARTIŞMA ve SONUÇ: Bu çalışmanın sonuçları, bu yağların özellikle kardiyovasküler hastalıklar üzerinde faydalı etkileri olan ve lipozomlar, nano ve mikroemülsiyonlar, sabunlar vb. üretiminde farmasötik ve kozmetik endüstrileri için önemli bileşikler olan linoleik ve oleik asitlerin değerli kaynakları olduğunu göstermiştir

**Anahtar Kelimeler:** yağ asitleri, gaz kromatografisi, datura, hyoscyamus, atropa,

## INTRODUCTION

*Hyoscyamus*, *Datura* and *Atropa* species are well known natural sources of tropane alkaloids, primarily scopolamine, atropine, and hyoscyamine. Due to their biological activity, these plants and substances are widely used in medicine as mydriatics, antiasthmatics, spasmolytics, etc. [1,2]. Furthermore, saponins, triterpenoids, phenolics, flavonoids, lignans, essential oils, sterols and other compounds were identified in the different organs of plants. [2-5]. Fatty acids are the main components of seed oils. The quality, consumption, industrial and medicinal uses of oils are mainly related to their fatty acids. According to various references, the percentage of oil differs from 15 to 35% in the seeds of these species [6,7].

Fatty acids have an essential role in the metabolic processes of human organisms - in the storage of energy, as basic components of cell membranes, etc. Scientists have reported that replacement of saturated fatty acids with monounsaturated and polyunsaturated fatty acids in the diet reduces the risk of cardiovascular disease by decreasing total and low-density lipoprotein cholesterol levels in the blood [7,8].

In the human body, linoleic acid is converted into docosahexaenoic (DHA) and eicosapentaenoic acids (EPA), which are responsible not only for the reduction of cholesterol, but also for inflammation, enhancement of brain functions, and prevention of cancers and autoimmune conditions [9]. Fatty acids are widely used in drug preparations as excipients and in the cosmetic industry to prepare soaps, fat emulsions, liposomes, etc. They are essential components in some drugs due to their biological activities [7, 10]. The seed oil of *D. stramonium* is used as an analgesic in neurological practice, as well as for hair removal in cosmetology [11]. Biodiesel was prepared with the fatty acids of some *Datura* species and suggested as an alternative fuel source [6, 12, 13]. A scar cream is prepared with a Swiss recipe by using seed oil of *H. niger* under the brand name - Kelosoft and successfully used and sold in Europe.

In the presented study, fatty acids of seed oils were analyzed in the accredited laboratory of the Food Safety Agency of the Republic of Azerbaijan.

## MATERIALS AND METHODS:

### *Plant collection*

Plants were collected in the middle or end of vegetation periods, from various regions of Azerbaijan (Table 1) Species were authenticated at the Institute of Botany of the Azerbaijan National Academy of Sciences, Baku, where voucher specimens are deposited.

Table 1. Collection times and places of plant species

No	Plants	Location	Time
1	<i>D.stramonium</i>	Baku	October 2019

		N 40°27'21.978" E 49°59'00.1752"	
2	<i>D.stramonium</i> var. <i>tatula</i>	Baku  N 40°27'3.6072" E 49°56'47.4576"	September 2018
3	<i>H.reticulatus</i>	Lerik – Zuvand  N 38°40'21.1548" E 48°21'31.9428"	June 2019
4	<i>H.niger</i>	Gusar  N 41°30' 21.528" E 48°14'35.5992"	July 2019
5	<i>A.caucasica</i>	Zagatala  N 41°35'31.8156" E 46°44'36.5748"	July 2019

#### *Chemicals and solvents*

Methanol, n-hexane and potassium hydroxide were purchased from Merck (Germany). The 0.45 µm membrane filters were purchased from Isolab (Germany). A standard mix of FAMEs (Supelco 37 Component FAME Mix, certified reference material, *TraceCERT*, in dichloromethane, ampule of 1 ml) was purchased from Sigma-Aldrich (Germany).

#### *Extraction of oil*

Seeds were dried and powdered. Extraction is carried out in the Soxhlet apparatus at 80°C for 6 hours. 10 g of raw materials and 300 ml of n-hexane were used to obtain seed oils. After solvent evaporation, the percentage of oil was calculated by mass (w/w).

#### *Preparation of fatty acid methyl esters*

Fatty acids are transferred to their volatile methyl esters (FAME) for GC analysis. 100 µl of oil, 5 ml of KOH-MeOH solution (0.5 M) were added into 10 ml glass tubes. The mixtures were heated at 60°C for 15 min. on the water bath under reflux, then centrifuged in the closed tubes at 900 rpm for 8 min. After cooling to room temperature, 5 ml of n-hexane and 5 ml of distilled water were added and mixed thoroughly. The n-hexane phases were collected, filtered (membrane filter 0.45 µm) and applied for analysis [14].

#### *GC conditions*

Analysis of fatty acids was performed on an Aligent 7820A GC system equipped with a FID (Flame ionization) detector and a capillary column HP-88 - 100 m × 0,25 mm × 0,25 µm. Helium was used as a carrier gas, with a flow rate of 1ml/min. The oven temperature was initially programmed at 120°C, hold 1 min. and increased to 175°C with the rate of 10°C/min., hold 10 min. Temperature was elevated to 210°C, hold 5 min. then 230°C, hold 5 min. with a rate of 5°C/min. The total run time was 37.5 min. The injector and FID temperatures were set at 250°C and 280°C, respectively. Split ratio was 1:50. The injection volume was 1 µl [15, 16].

#### *Standart sample*

The external standard was applied, which consists of 37 FAME components. A 10 mg standard mixture was added to the volumetric flask, diluted to 10 ml with n-hexane. The injection volume was 1 µl.

### *Results calculation*

The results were calculated in comparison with retention times ( $t_R$ ) of standard samples. The concentrations of fatty acids were calculated with peak areas and expressed as percentages. Each analysis was repeated three times and mean values were reported.

### **RESULTS**

The percentages of oils in the dried seeds are as follows: 22% in *D. stramonium*, 20% in *D. stramonium* var. *tatula*, 17% in *H. reticulatus*, 35% in *H. niger* and 32% in *A. caucasica*. Results are presented individually for each fatty acid of five plant samples in Table 2.

Linoleic (55-79%), oleic (11-26%), palmitic (4-12%) and stearic (2-3%) acids compose 97% of total fatty acids. Percentage of unsaturated fatty acids are 83-91%. Samples contain minor concentrations of *trans* fatty acids. Elaidic acid, a *trans* isomer of oleic acid (18:1t, <0.06%) and a *trans* isomer of linoleic acid (18:2t, <0.09%) are less than 1% of the total fatty acid mixtures. All other fatty acids are in the minor concentrations. The total numbers of fatty acids are varied - 28 in *D. stramonium*, 25 in *D. stramonium* var. *tatula*, 26 in *H. reticulatus*, 27 in *H. niger*, 23 in *A. caucasica*.

The highest yield of oil is obtained from *H. niger* seeds. The maximum concentration of linoleic acid is observed in the seed oil of *H. reticulatus* (79%). *D. stramonium* var. *tatula* contains a higher amount of oleic acid (26%) than other samples. Even *Datura* species contain 20-22% oils, they are one of the optimal sources of plant oil, linoleic and oleic acids, because a whole plant produce at least 200-300 g seeds. 100 g seeds were obtained in *Hyoscyamus* species, approximately 40 g seeds from *Atropa caucasica*. On the other hand, *Datura* species naturally grow in all regions of Azerbaijan. *Hyoscyamus* species were found in most parts of the country, but *Atropa caucasica* is rarely found in mountain forests.

*Table 2.* Concentration of all fatty acids in the seed oils of plants

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Nº	Fatty acids	t <sub>R</sub> , min.	<i>D. stramonium</i> , %	<i>D. stramonium</i> var. <i>tatula</i> , %	<i>H. reticulatus</i> , %	<i>H. niger</i> , %	<i>A. caucasica</i> , %
1	C4:0	4.43	0.02	0.07	0.00	0.06	0.00
2	C6:0	4.83	0.02	0.00	0.00	0.00	0.00
3	C8:0	5.46	0.02	0.00	0.00	0.00	0.00
4	C10:0	6.42	0.02	0.00	0.02	0.06	0.02
5	C12:0	7.77	0.02	0.02	0.07	0.03	0.01
6	C13:0	9.17	0.00	0.00	0.02	0.00	0.01
7	C14:0	9.55	0.15	0.12	0.06	0.05	0.03
8	C15:0	10.67	0.02	0.02	0.02	0.03	0.01
9	15:1	11.42	0.02	0.00	0.02	0.00	0.00
10	16:0	12.04	12.42	12.35	4.68	4.47	4.99
11	16:1	13.00	0.39	0.36	0.14	0.04	0.06
12	17:0	13.69	0.07	0.06	0.05	0.05	0.04
13	17:1	14.76	0.05	0.06	0.06	0.03	0.02
14	18:0	15.91	2.29	2.83	2.17	3.05	3.23
15	18:1t	16.84	0.05	0.03	0.06	0.03	0.01
16	18:1c	17.14	18.38	26.98	11.65	17.34	12.23
17	18:2t	18.62	0.06	0.04	0.05	0.08	0.05
18	18:2c	19.15	64.52	55.67	79.00	72.35	77.25
19	20:0	20.44	0.27	0.35	0.31	0.34	0.18
20	18:3n6	21.32	0.04	0.02	0.02	0.03	0.35
21	20:1	21.43	0.12	0.09	0.17	0.23	0.11
22	18:3n3	22.40	0.02	0.02	0.03	0.04	0.01
23	21:0	22.96	0.02	0.02	0.08	0.08	0.07

24	22:0	24.20	0.17	0.14	0.30	0.21	0.05
25	20:3n3	26.14	0.02	0.02	0.04	0.03	0.05
26	23:0	26.36	0.06	0.05	0.02	0.03	0.00
27	20:4	26.86	0.00	0.00	0.00	0.03	0.00
28	24:0	28.31	0.09	0.11	0.15	0.13	0.03
29	20:5n3	28.81	0.14	0.06	0.08	0.21	0.00
30	24:1	29.34	0.00	0.02	0.00	0.05	0.00
31	22:6n3	32.81	0.03	0.03	0.04	0.16	0.09
Unsaturated FAs	-	83.8	83.4	91.4	90.6	90.2	
	Saturated FAs	-	15.6	16.1	8.0	8.6	8.6
	Total numbers of FAs	-	28	25	26	27	23

## DISCUSSION

The selected method, Gas chromatography flame ionization detection (GC-FID) is a useful and sensitive method for analyzing numerous compounds in a single run. Without wasting a long time, GC-FID method is successfully applied for routine analysis of fatty acids [17].

Results of previous studies showed that 50-70% of polyunsaturated *cis*-linoleic acid (18:2c), 15-30% of monounsaturated oleic acid (18:1c) and 2-7% of saturated palmitic (16:0) and stearic acids (18:0) were found as major compounds in the seed oils of *Hyoscyamus* and *Datura* species [8, 11-9, 15, 18]. 65% of monounsaturated fatty acids were reported by Koria and Nithya in the seed oil of *D. stramonium* grown in India. Contrary to other studies, polyunsaturated fatty acids (18%) were lower than monounsaturated fatty acids [11,12]. Eicosanedicarboxylic acid -20:0 (34.55%), isomers of linoleic acid, 18:2n7 (4.56%) and 18:2n8 (3.61%), and eicosatrienoic acid 20:3n6 (4.39%), saturated - daturic acid C<sub>17</sub>H<sub>34</sub>O<sub>2</sub> were reported in *D. stramonium* oil [11].

Similar results were acquired in comparison with previous investigations. The total numbers of fatty acids are maximum in presented study.

## CONCLUSIONS

The investigation evaluated the presence and concentrations of 37 fatty acids in five plant species from Azerbaijan. *Datura*, *Hyoscyamus* and *Atropa* species aren't harvested widely as oil and unsaturated fatty acid sources due to their toxicity. To the best of our knowledge, this is the first study of fatty acids in *A. caucasica*.

Depending on geographical and climate differences, components of the fatty acid mixture and their concentrations could be varied in the same species. An essential omega-6 fatty acid – linoleic acid dominates all investigated samples and gives them valuable nutritional and medicinal properties. 2<sup>nd</sup> major compound of oils is oleic acid. Another essential omega-3 fatty acid – linolenic acid, is found in trace amounts. Changes of 4 main fatty acids concentrations are presented in Figure 1.

The results of the investigation might be useful for future researchers to evaluate these oils in medicinal, nutritional, cosmetic, fuel and other industries.

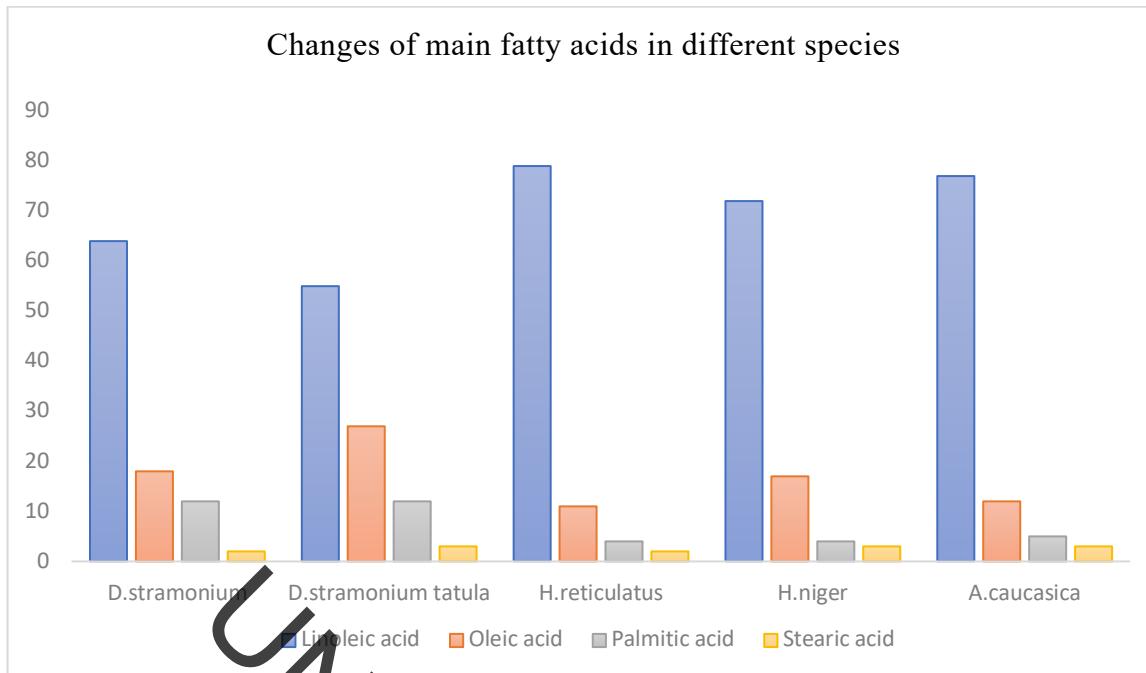


Figure 1. Concentrations (%) of 4 fatty acids in plant species

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