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ORIGINAL ARTICLE

Analysis of Fatty Acids of Some *Hyoscyamus*, *Datura*, and *Atropa* Species from Azerbaijan

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ABSTRACT

Objectives: *Datura stramonium* L., *D. stramonium* var. *tatula* (L.) Torr., *Hyoscyamus reticulatus* L., *H. niger* L., and *Atropa caucasica* Kreyer naturally found in Azerbaijan and their seeds possess 17-35% of oils. This study aims to evaluate and determine fatty acids of these plants special to Azerbaijan climate and geography. The presented study is the first to research into fatty acids of *A. caucasica*, which is an endemic species of Caucasus.

Materials and Methods: Fatty acid seed oils were derivatized to methyl esters and analyzed by gas chromatography equipped with a flame ionization detector, compared 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 two *trans*-fatty acids, were determined in the samples. Significantly high concentration of a medicinally important polyunsaturated fatty acid, *e.g.* linoleic acid, was observed in all samples.

Conclusion: The results of this study showed that these oils are particularly 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*.

Key words: Fatty acids, gas chromatography, *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 have been 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 play 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 the 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 and eicosapentaenoic acids, 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

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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 *Datura 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 *Hyoscyamus niger* under the brand name, *i.e.* Kelosoft®, and successfully sold in Europe.

In the present 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 their voucher specimens were deposited (date: 01.09.2020, no: 01.04/1191).

Chemicals and solvents

Methanol, *n*-hexane and potassium hydroxide were purchased from Merck (Germany). 0.45 µm membrane filters were purchased from Isolab (Germany). A standard mix of fatty acid methyl esters (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

The seeds were dried and powdered. Extraction was carried out in a Soxhlet apparatus at 80°C for 6 h. 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

FAMEs for gas chromatography (GC) analysis. The oils (100 µL for each) and 5 mL of KOH-MeOH solution (0.5 M) were added to 10 mL-glass tubes. The mixtures were heated at 60°C for 15 min in 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 to analysis.[14]

Gas chromatography conditions

Analysis of fatty acids was performed on an Aligent 7820A GC system equipped with a flame ionization detection (FID) detector and a capillary column HP-88 - 100 m × 0.25 mm × 0.25 µm. Helium was used as carrier gas with a flow rate of 1 mL/min. 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 and 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. The split ratio was 1:50. The injection volume was 1 µL.[15,16]

Standard sample

A standard mixture, which consists of 37 FAME components was applied. 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 compared with the retention times of standard samples. The concentrations of fatty acids were calculated using peak areas and expressed as percentages. Each analysis was repeated three times and mean values were reported. Statistical analysis has not been performed for evaluation of the results.

RESULTS

The percentages (w/w) 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%. Changes of four main fatty acid concentrations are presented in Figure 1. Samples contained minor concentrations of *trans*-fatty acids.

Table 1. Collection times and places of plant species

No	Plant species	Location	Time of collection
1	*Datura stramonium*	Baku N 40°27'21.978" E 49°59'00.1752"	October, 2019
2	*Datura stramonium* var. *tatula*	Baku N 40°27'3.6072" E 49°56'47.4576"	September, 2018
3	*Hyoscyamus reticulatus*	Lerik - Zuvand N 38°40'21.1548" E 48°21'31.9428"	June, 2019
4	*Hyoscyamus niger*	Gusar N 41°30' 21.528" E 48°14'35.5992"	July, 2019
5	*Atropa caucasica*	Zagatala N 41°35'31.8156" E 46°44'36.5748"	July, 2019

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

No	Fatty acids	t_R, min	*Datura stramonium*, %	*Datura stramonium* var. *tatula*, %	*Hyoscyamus reticulatus*, %	*Hyoscyamus niger*, %	*Atropa caucasica*, %
1	C4:0	4.43	0.02	0.07	-	0.06	-
2	C6:0	4.83	0.02	-	-	-	-
3	C8:0	5.46	0.02	-	-	-	-
4	C10:0	6.42	0.02	-	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	-	-	0.02	-	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	C15:1	11.42	0.02	-	0.02	-	-
10	C16:0	12.04	12.42	12.35	4.68	4.47	4.99
11	C16:1	13.00	0.39	0.36	0.14	0.04	0.06
12	C17:0	13.69	0.07	0.06	0.05	0.05	0.04
13	C17:1	14.76	0.05	0.06	0.06	0.03	0.02
14	C18:0	15.91	2.29	2.83	2.17	3.05	3.23
15	C18:1 *t*	16.84	0.05	0.03	0.06	0.03	0.01
16	C18:1 *c*	17.14	18.38	26.98	11.65	17.34	12.23
17	C18:2 *t*	18.62	0.06	0.04	0.05	0.08	0.05
18	C18:2 *c*	19.15	64.52	55.67	79.00	72.35	77.25
19	C20:0	20.44	0.27	0.35	0.31	0.34	0.18
20	C18:3, n6	21.32	0.04	0.02	0.02	0.03	0.35
21	C20:1	21.43	0.12	0.09	0.17	0.23	0.11
22	C18:3, n3	22.40	0.02	0.02	0.03	0.04	0.01
23	C21:0	22.96	0.02	0.02	0.08	0.08	0.07
24	C22:0	24.20	0.17	0.14	0.30	0.21	0.05
25	C20:3, n3	26.14	0.02	0.02	0.04	0.03	0.05
26	C23:0	26.36	0.06	0.05	0.02	0.03	-
27	C20:4	26.86	-	-	-	0.03	-
28	C24:0	28.31	0.09	0.11	0.15	0.13	0.03
29	C20:5, n3	28.81	0.14	0.06	0.08	0.21	-
30	C24:1	29.34	-	0.02	-	0.05	-
31	C22:6, n3	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 number of FAs	-	28	25	26	27	23

t_R: Retention times

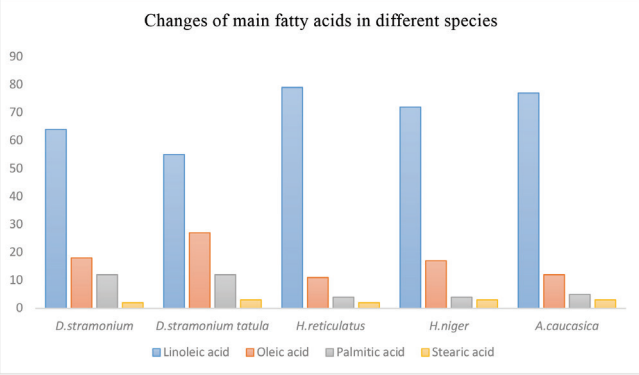

Figure 1. Concentrations (%) of four fatty acids in the plant species analyzed

Elaidic acid, a *trans* isomer of oleic acid (C18:1 *t*, <0.06%) and a *trans* isomer of linoleic acid (C18:2 *t*, <0.09%) are less than 1% of the total fatty acid mixtures. All other fatty acids are in the minor concentrations. The total number of fatty acids are varied - 28 in *D. stramonium*, 25 in *D. stramonium* var. *tatula*, 26 in *H. reticulatus*, 27 in *H. niger*, and 23 in *A. caucasica.*

The highest yield of oil was 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. On the other hand, *Datura* species contain 20-22% oils as they are one of the optimal sources of plant oil, linoleic, and oleic acids because a whole plant produces at least 200-300 g seeds. 100 g of seeds were obtained from *Hyoscyamus* species, while approximately 40 g of seeds were gathered from *A. caucasica.* However, *Datura* species naturally grow in all regions of Azerbaijan. *Hyoscyamus* species were found in most parts of the country, but *A. caucasica* is rarely found in mountain forests.

DISCUSSION

The selected method, 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 (C18:2), 15-30% of monounsaturated oleic acid (C18:1) and 2-7% of saturated palmitic (C16:0) and stearic acids (C18:0) were found as major compounds in the seed oils of *Hyoscyamus* and *Datura* species.[8,9,11,15,18] 65% of monounsaturated fatty acids were reported by Koria and Nithya[12] 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] Eicosanoic acid (C20:0) (34.55%), isomers of linoleic acid, (C18:2, n7) (4.56%) and (C18:2, n8) (3.61%), and eicosatrienoic acid (C20:3, n6) (4.39%), saturated - daturic acid $C_{17}H_{34}O_2$ were reported in *D. stramonium* oil.[11] Similar results were acquired compared with previous investigations. Total number of fatty acids was maximum in the present study.

CONCLUSION

The investigation evaluated the presence and concentrations of 37 fatty acids in five plant species from Azerbaijan. *Datura, Hyoscyamus,* and *Atropa* species are not harvested widely as oil and unsaturated fatty acid sources due to their toxicity. To the best of our knowledge, this is the first study on 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. Second major compound of oils is oleic acid. Another essential omega-3 fatty acid - linolenic acid, is found in trace amounts.

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

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Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.V., Design: A.V., Data Collection or Processing: A.V., A.K., N.H., Analysis, or Interpretation: A.V., A.K., N.H., Literature Search: A.V., A.K., N.H., Writing: A.V., E.G.

Conflict of Interest: No conflict of interest was declared by the authors.

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