Herbal Medicines Journal **2023; in press**
ISSN: 2538-2144

Short Communication

A Quantitative and Qualitative Study of Fatty Acids in *Ziziphus* Species in Iran

Maryam Keshavarzi[1]*, Kefayat Sharifat[1], Faezeh Fazeli[2]

[1]Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2]Department of Biological Sciences, Shahid Rajaee Teacher Training University, Tehran, Iran

Received: 09.11.2022; Accepted: 19.12.2023

Abstract

The genus *Ziziphus* belongs to the Rhamnaceae family with 70 species of trees in the world. There are three native species of this genus in Iran. The fruit of these trees is edible and has medicinal value. *Ziziphus* samples were gathered from natural habitats in Iran. We used cold methylation and gas chromatography to extract and identify of fatty acids. Identification of the fatty acids was carried out through the investigation of the curves. The significance of the quantitative results was evaluated using one-way analysis of variance. The study of fatty acids in pulps and seeds of the *Ziziphus* species showed that Oleic acid was the indicator in the studied species. Differences in the type of fatty acid were observed between the species. Examination of the fatty acids in the *Ziziphus* seed revealed that oleic acid is the main constituent of this oil and there is a quantitative and qualitative difference in the fat of the species. The fatty acids in seeds are higher than the fatty acids in the fruit pulp and the percentage of unsaturated fatty acids is higher than that of saturated fatty acids. The findings of this study are consistent those of with previous studies.

Keywords: Fatty acids, Iran, Rhamnaceae, *Ziziphus*

***Corresponding Author:** Maryam Keshavarzi, Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, (+98) 9122892720 - Tel/Fax: (+98) 88058912; Email: M.keshavarzi@alzahra.ac.ir.

Please cite this article as: Keshavarzi M, Sharifat K, Fazeli F. A Quantitative and Qualitative Study of Fatty Acids in *Ziziphus* Species in Iran. Herb. Med. J. 2023;in press.

Introduction

The genus *Ziziphus* L. is found in tropical and subtropical regions of the world with about 70 species of trees or small-leaved shrubs (1). Some species such as *Z. mauritiana* and *Z. jujuba* are scattered in all parts of the world (2). Different species of this genus have edible fruits and medicinally valuable compounds (3-5). These compounds are used in food and cosmetics industries. The fruit is a drupe with a juicy mantle and a hard wooden core.

Guil-Guerrero *et al*. (2004) investigated the presence of fatty acids and carotenoids in some varieties of *Z. jujuba* Mill (6). In 2012, Aloui and colleagues examined the fatty acids and oil composition of the seeds of four Tunisian ecotypes of *Ziziphus* (7). Using the Gas Chromatography (GC) method, these researchers found some differences in the studied species. The oil isolated from the seeds of *Z. spina-christi* was investigated. The comparison of four populations from Tunisia showed similarity in the qualitative profiles of fatty acids (7). These researchers identified oleic acid as the main component of seed oil in two *Ziziphus* ecotypes. Five main sterols were identified, and β-sitosterol was the most important constituent in all the ecotypes (7).

The composition of fatty acids in the fruit of *Ziziphus lotus* L. (Desf.) in Tunisia and the diversity in the biological activities of leaf and fruit extracts were investigated by Ghazghazi *et al*. in 2014 (8). The results obtained by these researchers revealed that the main components of fat in this species were the oleic acid and elaidic acid.

There are three native species of *Ziziphus* in Iran, including *Z. nummularia*, *Z. spina-christi* and *Z. mauritiana*. In the present study, the fatty acid profiles of the fruit pulp and the seeds of these species were investigated in order to evaluate their nutritional and medicinal values.

Materials and Methods

Sample Preparation

Fruit samples of *Ziziphus* species were gathered from various regions of Iran in the growing season of 2014 and 2015 (Table 1). The samples were identified, and then the separated fruit pulp and core were weighed and used for fatty acid extraction without pretreatment. Five grams of both *Z. spina-christi* and *Z. nummularia* species and 2.70 grams of *Z. mauritiana* seeds were extracted. The ratio of kernel to fruit weight was measured. By breaking the seed kernel, it was separated and placed in the oven for 24 hours to dry completely. The crushed seeds and fruit pulp were wrapped in filter paper, weighed and used for oil extraction. The extraction was based on the method developed by Folch *et al*. (1957) (9).

Fatty acid Extraction

Fatty acid extraction was done using Soxhlet (PSU-500). In the extractor part, the sample was exposed to N-hexane solvent with filter paper so that the solvent would be above the surface of the sample. Fatty acid extraction lasted three hours for each sample and was done under reduced pressure and 60°C temperature. The extracted material was stored at 4°C. For quantitative analysis, the glasses containing fatty acid were exposed to air for one night to remove N-hexane. At the bottom of the jars, a thick layer of fatty acid remained, and its weight was measured.

Methylation of Fatty Acids

Methylation of fatty acids and analysis of their methylated esters were performed according to ISO standards (Table 2 and Fig. 1). Methylation was done by the trans-esterification method. To extract fatty acids, a cold methylation method with 2 M alcohol potassium solution was used, and 2 drops of oil were mixed with 3 ml of 2 M ethanol (methanol) and 5 ml of normal heptane (methylation time 30 minutes). After 5 minutes, this fatty acid compound was methylated, and from the upper phase 0.5 microliters of the mixture was removed and injected into the GC device (Youngling 6100). Details of the device conditions were as follows: Column type: capillary column - BPX70 SGE, with a diameter of 0.5 mm and a length of 60 meters. The thickness of inner layer was 0.25 micrometers. Temperature conditions: temperature gradient varied from 150°C with an increase rate of 5°C/min to 190°C, maintaining the temperature in the final conditions for a period of time (total analysis time was 40 minutes). The type of carrier gas: hydrogen with a flow of 1 ml/min, Injector temperature: 280 degrees Celsius,

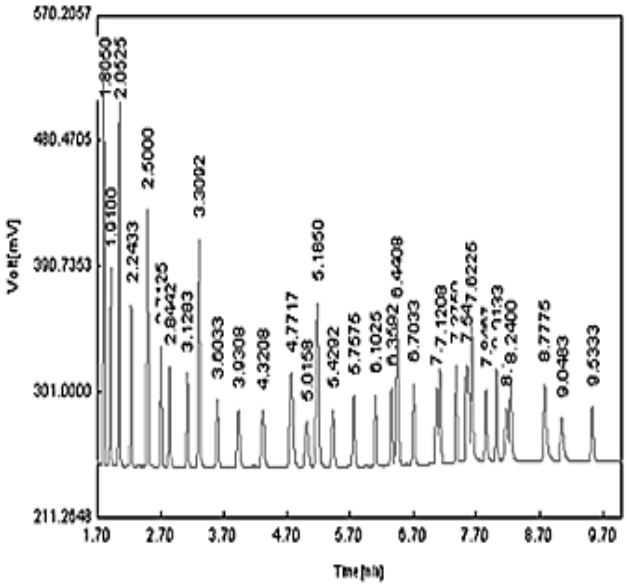

Figure 1. GLC Chromatogram of Standard Fatty Acids.

Table 1: Voucher Details of the Investigated *Ziziphus* Species.

Species	Location Detail
Z. spina -christi	Khuzestan, Mahshahr, Sharifat
Z. nummularia	Khuzestan, Andika, Adiv Mountain, fields near Chegarman, Nanaee
Z. mauritiana	Khuzestan, Abadan, Sharifat

Detector location temperature: 300 degrees Celsius.

Results and Discussion

The percentage of the total lipid obtained from the studied species has been shown in Table 3. Fatty acids of *Z. nummularia* include myristic acid, palmitic acid, heptadecanoic acid, cis-heptadecanoic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, palmitoleic acid, erucic acid, gadoleic acid, alpha-linolenic acid, trans- linolenic acid, linoleic acid, and oleic acid. The relevant chromatogram (Fig. 2) shows that the highest percentage of fatty acids in this species is related to oleic acid (Table 4).

Fatty acids obtained from *Z. spina-christi* include myristic acid, palmitic acid, margaric acid, cis-heptadecanoic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, palmitoleic acid, gadoleic acid, trans- linolenic acid, alpha- linolenic, linoleic acid, oleic acid, and dimethyl erucite (Fig. 3). The highest percentage of fatty acids was related to oleic acid (Table 5).

Fatty acids obtained from *Z. mauritiana* include myristic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, palmitoleic acid, cis-heptadeconic acid, gadoleic acid, trans-linolenic acid, alpha- linolenic acid, linoleic acid, oleic acid, pentadecylic acid, linolelaidic acid and elaidic acid (Fig. 4). The highest percentage of fatty acids was related to oleic acid (Table 6).

The results of the analysis of fat in the seeds revealed

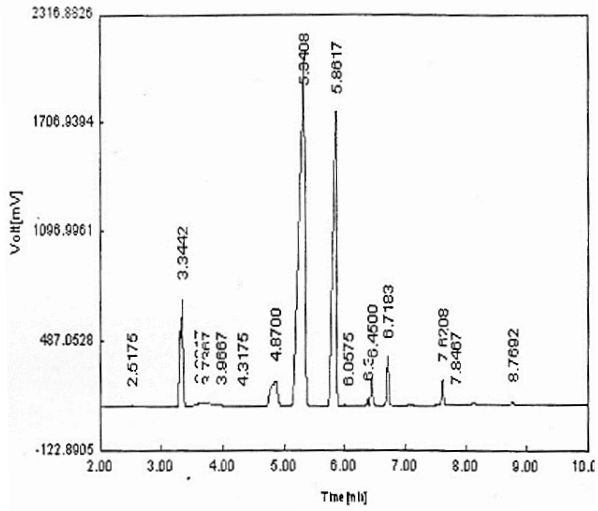

Figure 2. GC Chromatogram of Standard Fatty Acids in *Z. nummularia* Species.

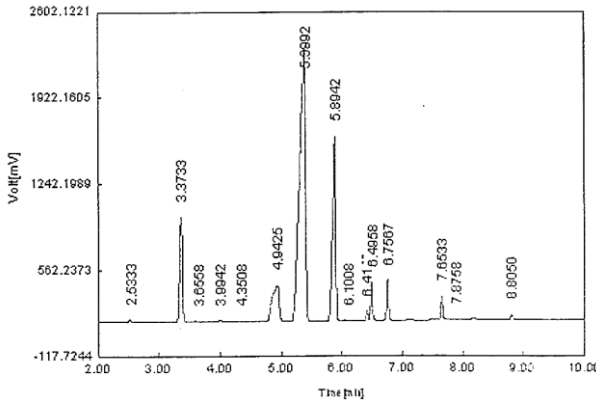

Figure 3. GLC Chromatogram of Standard Fatty Acids in *Z. spina-christi* Species.

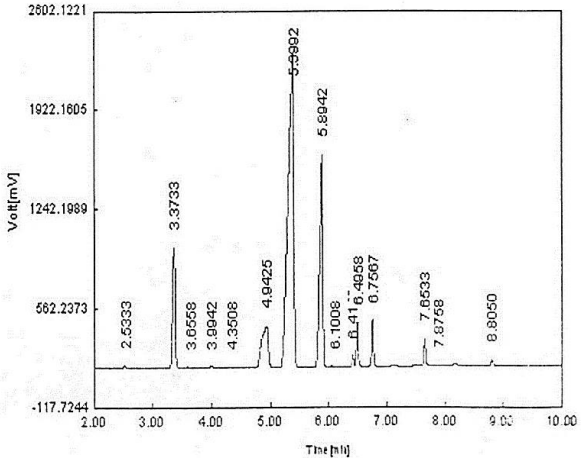

Figure 4. GLC Chromatogram of the Standard Fatty Acids in *Z. mauritiana* Species.

that *Z. nummularia* and *Z. mauritiana* had the highest and the lowest percentage of fatty acids in the studied species, respectively. A comparison of the amount of fatty acid in the fruit coat indicated that the highest amount of fatty acid was in *Z. numularia* species. The results of fatty acid analysis revealed that the percentage of unsaturated fatty acids was higher than that of saturated fatty acids in all three species, which is consistent with the findings of Makhdar *et al*. in 2019 who examined *Z. lotus* fruit oil (Table 7) (10).

These researchers reported the richness of unsaturated fatty acids (80%), a high percentage of which was related to oleic acid (66.8%) and 13.6% was related to linoleic acid. In 2019, Reche *et al*. also found that unsaturated fatty acids were more than saturated fats in their investigation of fatty acids in jujube fruit skin (*Z. jujube* Mill.) (11). Mousavimanesh *et al*. (2022) found that the same fatty acids were predominant ones in different *Lycium* species (12).

Table 2: The List of Standard Fatty Acids Used in the Present Study. Saturated (S)/ unsaturated (U).

Fatty acid	S/U	Retention time	Fatty acid	S/U	Retention time
Linoleic acid	U	5.7575	Caprylic acid	S	1.6683
Calendic acid	U	6.1025	Capric acid	S	1.8050
Rumelenic acid	U	6.3592	Undecyclic acid	S	1.9100
Arachidic acid	S	6.4408	Lauric acid	S	2.0525
Trans-Linoleic acid	U	6.7033	Tridecyclic acid	S	2.2433
Eicosenoic acid	U	7.0717	Myristic acid	S	2.5000
Dihomo-γ-linolenic acid	U	7.1208	Myristoleic acid	U	2.7125
Epoxyeicosatrienoic acid	U	7.3750	Pentadecyclic acid	S	2.8442
Arachidonic acid	U	7.5408	Pentadecenoic acid	U	3.1283
Behenic acid	S	7.6225	Palmitic acid	S	3.3092
Erucic acid	U	7.8467	Palmitoleic acid	U	3.6033
Eicosapentaenoic acid	U	8.0133	Margaric acid	S	3.9308
Tricocyclic acid	S	8.1758	Heptadecenoic acid	U	4.3208
Docosadienoic acid	U	8.2400	Stearic acid	S	4.7717
Lignoceric acid	S	8.7775	Elaidic acid	U	5.0158
Nervonic acid	U	9.0483	Linoleic acid	U	5.1850
Docosahexaenoic acid	U	9.5333	Linoelaidic acid	U	5.4292

In their investigation of the fruit of 21 different varieties of jujube, these researchers identified 24 types of fatty acids. The types of fatty acids and their contents were significantly different in cultivars. Some cultivars had high contents of palmitoleic acid. Most cultivars had high contents of oleic acid and linoleic acid (13), which is consistent with the findings of the present study. A high ratio of unsaturated/saturated fatty acids was observed in several cultivars, which is also consistent with the results of studies conducted on different species of *Ziziphus* in Iran. Values and nutritional properties of the cultivars were different based on the geographical region.

Among the saturated fatty acids, pentadecylic acid was found in *Z. mauritiana*, while it was not present in the other two species. Among the unsaturated fatty acids, elaidic acid was present only in *Z. mauritiana*. Moreover, erucic acid was present in a small amount in *Z. spina-christi*, while it was not present in the other

Table 3: The Percentage of the Total Lipid in the Studied Species.

Species	Seed	Pulp
Z. nummularia	28.5	13.62
Z. spina-christi	23.46	4.27 8
Z. mauritiana	16.55	5.58

Table 4: The Percentage of Fatty Acids in *Z. nummularia* Species.

Fatty acid	Amount (% g.)
Myristic acid	0.0516
Palmitic acid	8.1026
Palmitoleic acid	0.6682
heptadecanoic acid	0.0497
Cis-heptadecanoic acid	0.0651
Stearic acid	4.4959
Oleic acid	53.3529
Linoleic acid	27.7900
Trans-Linolenic acid	0.0101
alpha-Linolenic acid	0.2306
Arachidic acid	1.3817
Eicosenoic acid	2.2115
Behenic acid	1.2014
Erucic acid	0.0558
Lignoceric acid	0.3000

Table 5: The Percentage of Fatty Acids Obtained from *Z. spina-christi*. Species Compared with the Set of Fatty Acids.

Fatty Acid	Amount (% g.)
Tetradecanoic acid	0.1349
Palmitic acid	9.6040
Palmitoleic acid	0.1152
heptadecanoic acid	0.1181
Cis-heptadecanoic acid	0.0501
Stearic acid	8.3523
Oleic acid	54.5560
Linoleic acid	19.7150
Trans-Linolenic acid	0.0582
alpha-Linolenic acid	0.4858
Arachidic acid	2.4774
Eicosenoic acid	2.3090
Behenic acid	1.4912
Erucic acid	0.0499
Lignoceric acid	0.4829

two species.

Since the fatty acids in the seeds were more than fruit pulp, more investigations have been carried out on the chemical composition of the seeds. In all the three studied species, the percentage of unsaturated fatty acids was higher than saturated fatty acids. The highest amounts of saturated and unsaturated fatty acids in all the three species studied were related to palmitic acid and oleic acid, respectively. Pentadecylic acid was found in *Z. mauritiana*, while it was absent in the other two species. Elaidic acid was present in *Z. mauritiana*, while it was not observed in the other two species.

In the study conducted by Aloui *et al*. in 2012 on the fatty acids and oil composition of the seeds of four Tunisian ecotypes of *Ziziphus* (7), the presence of quantitative changes in the resulting composition was determined and similarity was observed in the qualitative profiles. These researchers identified oleic acid as the main composition of oil in several ecotypes, although in an ecotype called Shutrana, the fat was rich in linoleic acid (7, 8).

Conclusion

The findings of the present study also confirm the prominent role of the Oleic acid in the main composition of seed fat. This fatty acid is considered valuable in the industry to produce soap and cosmetics. The results of the present research confirm that different species of *Ziziphus* in Iran have useful and valuable fatty acids that can be used in food and agriculture industries.

Table 6: The Percentage of Fatty Acids Obtained from the *Z. mauritiana*. Species Compared with the Set of Fatty Acids.

Fatty acid	Amount (% g.)
Tetradecanoic acid	1.0020
pentadecanoic acid	0.4523
Palmitic acid	11.8450
Palmitoleic acid	1.5075
heptadecanoic acid	0.1623
Cis-heptadecanoic acid	0.2040
Stearic acid	7.6852
Elaidic acid	0.9504
Oleic acid	43.5585
Linolelaidic acid	0.1842
Linoleic acid	25.0127
Trans-Linolenic acid	0.1179
alpha-Linolenic acid	0.4771
Arachidic acid	2.3887
Eicosenoic acid	2.1762
Behenic acid	1.6575
Lignoceric acid	0.5605

Table 7: Significant Fatty Acids in the Studied Species and the percentage ratio of saturated to unsaturated fatty acids. The Main Fatty Acid was Oleic Acid.

Species	PSFA	PUFA	SUR
Z. nummularia	15.5829	85.09	5.460
Z. mauritiana	35.7535	74.1233	2.8781
Z. spina-christi	22.6608	77.2539	3.4091

PSFA: Percentage of Saturated Fatty Acids, PUFA: Percentage of Unsaturated Fatty Acids, SUR: Saturated to unsaturated Ratio.

Acknowledgment

The present research did not receive any specific grant from public funding agencies, commercial, or not-for-profit sectors. The vice-chancellor of research of Alzahra University financially supported this work. The authors acknowledge anonymous reviewers for their relevant comments.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

None.

References

1. POWO. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published at: http://www.plantsoftheworldonline.org [Accessed 9 March. 2022].
2. Asare M. Biological characteristics of *Ziziphus* trees in Iran, Forest and Rangeland Research Institute, Tehran (In Persian). 2008.
3. Tripathi S. *Ziziphus jujuba*: A phytopharmacological review. Int J Res Dev Pharm Life Sci. 2014; 3: 959-66.
4. Saad AH, Kadhim RB, Rasool BA. Formulation and evaluation of herbal shampoo from *Ziziphus spina-christi* leaves extract. Int J Res Ayurveda Pharm. 2011; 2:1802-6.
5. Rathore SK, Bhatt SH, Dhyani S, Jain A. Preliminary phytochemical screening of medicinal plant *Ziziphus mauritiana* Lam. fruits. Int J Curr Pharm Res. 2012; 4:160-2.
6. Guil-Guerrero JL, Delgado A, Gonzalez MC, Isasa MT. Fatty acids and carotenes in some ber (*Ziziphus jujuba* Mill) varieties. Plant Foods Hum Nutr. 2004; 59:23-7.
7. Aloui ME, Mguis K, Laamouri A, Albouchi A, Cerny M, Mathieu C, Vilarem G, Hasnaoui B. Fatty acid and sterol oil composition of four Tunisian ecotypes of *Ziziphus zizyphus* (L.) H. Karst. Acta Bot Gall. 2012; 159:25-31.
8. Ghazghazi H, Aouadhi C, Riahi L, Maaroufi A, Hasnaoui B. Fatty acids composition of Tunisian *Ziziphus lotus* L.(Desf.) fruits and variation in biological activities between leaf and fruit extracts. Nat Prod Res. 2014; 28:1106-10.
9. Folch J, Lees M, Stanley GHSA. A simple method for total lipid extraction and purification. J Biol Chem. 1957;226(1), 497-509.
10. Makhdar N, Anouar A, Bouyazza L. Composition in fatty acids, sterols and tocopherols of vegetable oil extract from kernels of *Zizyphus lotus* L. J Mater Environ Sci. 2019; 10:1074-82.
11. Reche J, Almansa MS, Hernández F, Carbonell-Barrachina ÁA, Legua P, Amorós A. Fatty acid profile of peel and pulp of Spanish jujube (*Ziziphus jujuba* Mill.) fruit. Food chem. 2019; 295:247-53.
12. Mousavimanesh F., Keshavarzi M., Nazem Bokaee Z., Fazeli F. Fatty Acid Composition of *Lycium* (Solanaceae) Species. Herbal medicines Journal. 2022; 7(1).
13. Zhang R, Sun X, Vidyarthi SK, Wang F, Zhang Y, Pan Z. Characteristics of fatty acids in the Chinese jujube fruits (*Ziziphus jujuba* mill.). J Agric Food Res. 2021; 4:100129.

© **Maryam Keshavarzi, Kefayat Sharifat, Faezeh Fazeli**. Originally published in the Herbal Medicines Journal (http:/www.hmj.lums.ac.ir), 08.01.2024. This article is an open access article under the terms of Creative Commons Attribution License, (https://creativecommons.org/licenses/by/4.0/), the license permits unlimited use, distribution, and reproduction in any medium, provided the original work is properly cited in the Herbal Medicines Journal. The complete bibliographic information, a link to the original publication on http://www. hmj.lums.ac.ir/, as well as this copyright and license information must be included.