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Analysis of Fatty Acid Composition of Crude Seed Oil of *Lactuca sativa* L. by GC-MS and GC Methods

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Abstract

Lactuca sativa L. (Garden Lettuce), is an edible herb cultivated in Iran and other parts of the world. In traditional Iranian pharmacy books, garden lettuce is named "*Kass Bostāni*" and Hakim Aqili classified it as a "*Ghazā'ye Dawā*'ee" (*Ghazā* means Food; *Dawā* means Drug). It is said to be soporific, prescribed to cure insomnia and to be useful in thirst and feeling of hotness and burning in the stomach. Seeds of this herb reduce semen, suppress libido, and are useful in cases of frequent nocturnal emissions. The fixed oil obtained from seeds of this plant is reputed to have hypnotic and brain moistening properties. In this study, we aimed to analyze the fatty acid composition of the crude seed oil of *Lactuca sativa* L. Methyl esterification of the fatty acids was performed by the method of Ken'ichi Ichihara *et al*., but with a slight modification. Components of the oil were then extracted by n-hexane and analyzed by Gas chromatography-Mass spectroscopy and Gas Chromatography methods. The identified constituents, which represented 98.20% of the total elutes, were the methyl esters of linoleic (52.38%), oleic (34.42%), palmitic (7.25%), stearic (2.66%), arachidic (1.32%), and myristic (0.17%) acids. Total percentages of methyl esters of the saturated and unsaturated fatty acids identified in our examined oil were 11.4 and 86.80%, respectively. In conclusion, the seed fat of *Lactuca sativa* L., like many other plant fats, is rich in unsaturated fatty acids.

Keywords: Lactuca sativa, garden lettuce, seed oil, GC, GC-MS, fatty acids.

1. Introduction

Garden Lettuce, *Lactuca sativa* L. (Asteraceae) (1), is a well-known popular salad herb, cultivated and used in our country and other parts of the world. In traditional Iranian pharmacy books, lettuce is called "*Khass*" and described to be either wild ("*Khass barri*") or garden lettuce ("*Kass Bostāni*") (2-5). On the other hand, lettuce has been classified by Hakim Aqili as a "*Ghazā*'ye *Dawā*'ee" (*Ghazā* means Food; *Dawā* means Drug) (2).

Lettuce is said to be soporific, prescribed to cure insomnia and to be useful in thirst and feeling of hotness and burning in the stomach. Seeds of this herb reduce semen, suppress libido, and are useful in cases of frequent nocturnal emissions (4). The oil obtained from seeds of this plant is reputed to have hypnotic, anti-melancholic, anti-dry epilepsy (cures epilepsy caused by dryness), and anti-

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wine-bibbing properties. It has also brain moistening effect and is used in resolving hardness (2, 5).

As part of a research project designed for analysis of different parts and products of this plant, in this study, we aimed to analyze the fatty acid composition of the crude oil obtained from cold expression of its seeds. To the best of our knowledge, there is no report on analyzing the fatty acids of crude seed oil of *Lactuca sativa* L. by applying both Gas Chromatography-Mass Spectroscopy (GC-MS) and Gas Chromatography (GC) methods.

2. Materials and methods

2.1. Plant material

Seeds of *Lactuca sativa* L. were obtained from a commercial market in Isfahan, Iran, in 2016. They were cultivated in the Central Greenhouse of Isfahan University of Medical Sciences, and the fully developed plants were characterized by the Botany Department of Faculty of Sciences, University of Isfahan, Iran. Specimens of the flowering plant were deposited in the herbarium of medicinal plants of Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Iran under a voucher number 3405.

2.2. Fatty acid methyl esters and sample preparation

Methyl esterification of the fatty acids was performed by the method of Ken'ichi Ichihara *et al.* (6) with a slight modification. An appropriate amount of the seed oil was mixed with 5 ml of n-hexane and vortexed. Then, 500 µl of 2 M methanolic KOH was added and the mixture was shaken vigorously for 2 min and centrifuged. The upper hexane layer was then separated and stored in sealed vials at -18 °C before GC-MS and GC analyses.

2.3. GC-MS analysis

The components of the n-hexane extract obtained in the previous step were analyzed on an Agilent 7890A GC and Agilent 5975C mass detector under the following conditions: injection of 0.1µl samples, HP-5 MS capillary column (60 m×0.25 mm; film thickness 0.25 µm); carrier gas He, flow rate 1.3 ml/min, injector temperature 250 °C, temperature program: 40 °C hold for 3 min, 40-290 °C at 5 °C /min, then hold at 290 °C for 3 min; mass spectra: electronic impact, ionization potential 70 eV, ion source temperature 250 °C, ionization current 1000 µA, resolution 1000 and mass range 30-400.

Identification of the components, in this method, was performed by computational matching against the library spectra (library database Wiley 275.L) evluating their retention indices with reference to an n-alkane series in a temperature programmed run, interpreting the fragmentation pattern of the components, and comparison of their retention indices with the literature data (7,8).

2.4. GC analysis

Analysis of the components of the n-hexane extract was also carried out on an Agilent 6890 N under the following conditions: injection of 1µl samples, HP-88 MS capillary column (100 m× 0.25 mm; film thickness 0.20 µm); carrier gas nitrogen, flow rate 1 ml/min, injector temperature 260 °C, temperature program: 100 °C hold for 5 minutes, followed by 100-240 °C at 4 °C/min, then hold at 240 °C for 4 minutes.

Quantification of the components of the n-hexane extract eluted in the GC analysis was performed using standard methyl esters of the fatty acids in three replicate experiments.

3. Results and discussion

The GC spectrum of the fatty acid methyl esters of crude seed oil is shown in Figure 1, while the name of the identified constituents by GC-MS and their quantities, which were obtained by GC analysis representing 98.20% of the total elutes, are listed in Table 1.

The n-hexane extract of the examined oil was composed of a mixture of methyl esters of saturated and unsaturated fatty acids. Saturated fatty acids included the methyl esters of myristic acid (0.17%), palmitic acid (7.25%), stearic acid (2.66%), and arachidic acid (1.32%); while the unsaturated fatty acids composed of the methyl esters of linoleic (52.38%) and oleic (34.42%) acids.

The total percentage of methyl esters of the saturated fatty acids in our examined oil was

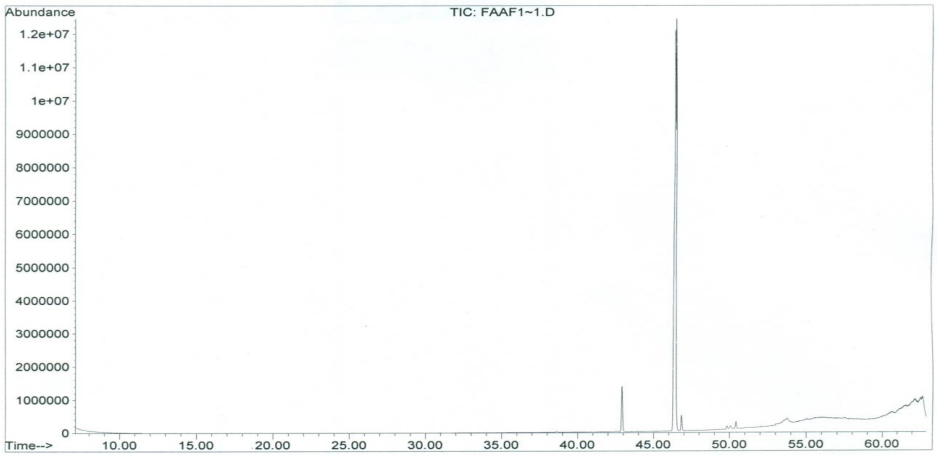

Figure 1. GC spectrum of the fatty acid methyl esters extracted by n-hexane from the crude seed oil of *Lactuca sativa* L. after esterification.

11.40%; while the total percentage of methyl esters of the unsaturated fatty acids (i.e: linoleic-or omega 6- and oleic acids) of our oil was 86.80%. Shaoqin *et.al.* (9), who extracted *Lactuca sativa* seed oil and its fatty acid composition by supercritical CO_2 and analyzed its fatty acid composition, have reported that the total percentage of the unsaturated fatty acid methyl esters of their oil was 86.68%. Harborne declared that oleic acid is often accompanied by di-unsaturated linoleic acid.

Table 1. Identified and quantified fatty acid methyl esters in n-hexane extract of crude seed oil of *Lactuca sativa* L.

No.	Components[a]	Calc. RI[b]	Rep. RI[c]	%[d]
1	Tetradecanoic acid methyl ester {Myristic acid methyl ester}	1725	1725	0.17
2	Hexadecanoic acid methyl ester {Palmitic acid methyl ester}	1930	1928	7.25
3	9,12-Octadecadienoic acid (Z,Z) methyl ester {Linoleic acid methyl ester}	2105	2111	52.38
4	9-Octadecenoic acid (Z) methyl ester {Oleic acid methyl ester}	2112	2116	34.42
5	Octadecanoic acid methyl ester (Stearic acid methyl ester)	2132	2127	2.66
6	Eicosanoic acid methyl ester {Arachidic acid methyl ester}	2332	2329	1.32
	Total:			**98.20**
	Saturated fatty acid methyl esters			**11.40**
	Unsaturated fatty acid methyl esters			**86.80**

[a]Components arranged in order of their elution from an HP-5MS capillary column.

[b]Retention indices (RI) calculated from retention times relative to those of C_5-C_{24} n-alkanes on HP-5MS column.

[c]Reported retention indices were extracted from references 7-8.

[d]Percentage of the components determined by GC method using an HP-88MS capillary column.

Meanwhile, the plant fats, unlike animal fats, are rich in unsaturated fatty acids and there is evidence that some of these fatty acids may be essential as a dietary requirement in man (10).

4. Conclusion

Lactuca sative L. seed fat, like many other plant fats, is rich in unsaturated fatty acids.

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Conflict of Interest

None declared.

5. References

1. Mozaffarian, V: Identification of Medicinal and Aromatic Plants of Iran. Tehran, Farhang Mo'aser Publishers, 2015.
2. Aqili MH: Makhzan Al' Advieh. Research, correction and annotation by: Shams Ardekani MR, Rahimi R, Farjadmand F. Tehran, Rahe Kamal with cooperation of Tehran University of Medical Sciences Publications, 2008.
3. Razi M: Al-Hāvi. Vol. 20. Translation, research and rearrangement of texts by: Afsharypuor,S. Tehran, Published by The Academy of Medical Sciences of I. R. Iran, 2005.
4. Bin Sina: AL-Qanun Fil-Tibb. Book 2. New Delhi, Printing Press, Hamdard Nagar, 1987.
5. Mo'men Tonekaboni M: Tohfat-ol-Mo'menin. Correction and research by: Rahimi R, Shams Ardekani MR, Farjadmand F. Tehran, Nashre Shahr, 2008.
6. Ken'ichi Ichihara Akira Shibahar, Kohei Yamamoto, Takao Nakayama. An improved method for rapid analysis of the fatty acids of glycerolipids. *Lipids*.1996; 31(5): 535-9.
7. Rostad C.E, Pereira W.E. Kovats and Lee retention indices determined by gas chromatography/mass spectrometry for organic compounds of environmental inerest. *HRC CC J High Resolut Chromatogr Chromatogr Commun.* 1986;9:328-34.
8. Ansorena D, Gimeno O, Astiasaran I, Bello J. Analysis of volatile compounds by GC-MS of a dry fermented sausage: chorizo de pamlona. *Food Res Int.* 2001;34:67-75.
9. Shaoqin H, Junping Z, Yili A, Hang B, Aisa H. Supercritical CO_2 extraction of *Lactuca sativa* seed oil and its fatty acid composition. *Zhongguo Youzhi*. 2015;40:1-5.
10. Harborne JB. Phytochemical Methods. Second Edition. London: Chapman and Hall, 1984.