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Short communication

Essential Oil Constituents of Seeds and Fresh Leaves of Garden Lettuce (*Lactuca sativa* L.) Grown in Isfahan, Iran

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Abstract

Background and objectives: Garden lettuce, *Lactuca sativa* L. (Asteraceae), is an herbaceous plant cultivated in different parts of Iran including Isfahan. In Iranian traditional medicine, it is classified as a medicinal food because it is nutritive and has therapeutic effects. As the therapeutic effects of the plant parts may be related to some volatile active constituents, it was aimed to determine the chemical constituents of the essential oils obtained from seeds and fresh leaves of the plant grown in Isfahan. **Methods:** Essential oils from seeds and fresh leaves of *Lactuca sativa* L. were obtained by hydro-distillation method and were analyzed by gas chromatography-mass spectroscopy (GC/MS) method. **Results:** Identified volatile constituents in the essential oils of the seeds and fresh leaves samples were representing 96.1% and 91.8% of the oils, respectively. Major volatile constituents of the seeds oil were: linoleic acid (38.8%), oleic acid (17.9%), palmitic acid (4.9%), 3-methyl heptane (3.2%), 1-decene (2.8%), 1-hexyl-3-methyl cyclopentane (2.3%), 2,4-decadienal (*E*,*E*) (2.2%), limonene (2.1%) and carvone (2.0%). While the main volatile constituents of the fresh leaves oil were: linoleic acid (37.7%), oleic acid (19.6%), palmitic acid (16.7%), 3-methyl heptane (4.6%), 1-ethyl-3-methyl cyclopentane (4.2%), 1-decene (2.0%), *n*-octadecane (1.7%) and *n*-tetradecane (1.5%). **Conclusion:** The essential oils from the seeds and fresh leaves of the plant grown in Isfahan mainly composed of a mixture of saturated and unsaturated fatty acids.

Keywords: aliphatic hydrocarbons; essential oil; fatty acids; garden lettuce; lettuce; *Lactuca sativa*

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Introduction

Garden Lettuce, Lettuce, *Lactuca sativa* L., belonging to Asteraceae family [1], is a well-known herb named "*Khass bostani"* in the Iranian traditional medical books [2-4]. It is cultivated in different parts of Iran including Isfahan, Khuzestan, Tehran, Gilan, Golestan, (Gorgan) and Mazandaran provinces [1]. This herb is glabrous, annual or biennial, has slender taproot and erect flowering stems 30-70 (-100) cm. Its basal leaves are undivided and shortly petiolate. The inflorescence is dense, and the heads are numerous, with 7-15 (-35) florets. The ligules are pale yellow, often violet-streaked. The seeds are 6-8 mm, grayish, and their body is obovate, 5 to 9 ribbed, often finely muricate at apex [1,5].

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In Iranian traditional medicine, garden lettuce "*Khass bostani*" is classified as a medicinal food because it is nutritive and has therapeutic effects [4]. According to suggestions of Rhazes [2], Avicenna [3] and Aghili [4], lettuce is cold and moist tempered and its coldness appears to be in the second degree. It is useful in thirst, and feeling of hotness and burning in the stomach. Lettuce is also soporific, cures insomnia when taken in fresh or boiled form, and is a drug meant for removing the obstructions in the nose. Seeds of this plant reduce semen, suppress libido and are useful in cases of frequent nocturnal emissions.

To the best of our knowledge, there has not been any report on essential oil constituents of *Lactuca sativa* L. grown in different parts of Iran including Isfahan. The therapeutic effects of the plant parts may be related to some volatile active constituents; therefor, as a part of a detailed project, in this study we decided to elucidate the volatile constituents of the seeds and fresh leaves essential oils of the plant grown in Isfahan using GC/MS method.

Material and Methods

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, Isfahan, Iran and the fully developed plants were characterized by the Botany Department of Faculty of Sciences, University of Isfahan, Isfahan, Iran. Voucher specimens (No. 3405) were deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Sample preparation

Seeds (50 g) and fresh leaves (150 g) were subjected separately to hydrodistillation with distilled water for 4 h using a Clevenger type apparatus. The volatile oils were collected in *n*-hexane, concentrated and stored in sealed vials at low temperature before GC/MS analysis.

GC/MS analysis

Determination of volatile constituents was performed on an Agilent 7890A GC and Agilent 5975C mass detector under the following conditions: injection of 0.1 μL samples, HP-5 MS capillary column (30 m×0.25 mm; film thickness 0.25 μm); carrier gas He, flow rate 2 mL/ min, injector temperature 250 °C, temperature program: 60-275 °C at 4 °C/ 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 volatile components

Identification of the components was performed by computer matching against the library spectra (library database Wiley 275.L), their retention indices with reference to an *n*-alkane series in a temperature programmed run, interpreting their fragmentation pattern and comparison of the mass spectra with the literature data [6-14].

Results and Discussion

Identified volatile constituents in the essential oils of the seeds and fresh leaves samples which were representing 96.1% and 91.8% of the oils, respectively have been shown in table 1.

Major volatile constituents of the seeds oil were: linoleic acid (38.8%), oleic acid (17.9%), palmitic acid (4.9%), 3-methyl heptane (3.2%), 1-decene (2.8%), 1-hexyl-3-methyl cyclopentane (2.3%), 2,4-decadienal (*E,E*) (2.2%), limonene (2.1%) and carvone (2.0%).

Main volatile constituents of the fresh leaves oil were: linoleic acid (37.7%), oleic acid (19.6%), palmitic acid (16.7%), 3-methyl heptane (4.6%), 1-ethyl-3-methyl cyclopentane (4.2%), 1-decene (2%), *n*-octadecane (1.7%) and *n*-tetradecane (1.5%).

Few other fatty acids (namely myristic and stearic acids), as well as aliphatic unbranched and branched saturated hydrocarbons, aliphatic branched unsaturated hydrocarbons, cyclopentane and cyclohexane derivatives, a furan derivative, naphthalene, aldehydes, ketones, alcohols, and terpenoids could be also detected in one or both oils (table 1).

Total amounts of saturated and unsaturated fatty acids in both seeds and fresh leaves oils were 62.1% and 74.6%, respectively**.** Harborne has declared that palmitic acid, which is a C_{16} acid, constitutes the major saturated acid in leaf lipids [15]. This phenomenon can be seen in our examined leaf oil when we compare the amount of palmitic acid (16.7%) with small amount of stearic acid (table 1).

Table 1. Essential oils constituents of seeds and fresh leaves of *Lactuca sativa* L. grown in Isfahan (Iran)

No.	Components[a]	Calc. RI[b]	Rep. RI[c]	% in seeds	% in fresh leaves
1	3-Methyl heptane	772	774	3.2	4.6
2	1,4-Dimethyl cyclohexane	783	779	tr[d]	tr
3	1-Ethyl-3-methyl cyclopentane	795	793	tr	4.2
4	n-Octane	800	800	0.2	0.1
5	4-Octene(*Z*)	809	808	-	tr
6	Propyl cyclopentane	836	832	0.7	tr
7	Ethyl cyclohexane	838	833	tr	tr
8	n-Nonane	900	900	tr	tr
9	Heptanal	902	902	tr	-
10	5-Methyl nonane	961	960	1.1	tr
11	3- Methyl nonane	972	971	1.5	1.0
12	1-Decene	989	990	2.8	2.0
13	2-n-Pentyl furan	993	998	1.9	-
14	n-Decane	1000	1000	0.3	0.5
15	α-Phellandrene	1005	1003	tr	-
16	Limonene	1032	1029	2.1	-
17	Benzene acetaldehyde	1044	1042	-	tr
18	2-Octenal(*E*)	1061	1060	0.3	-
19	n-Undecane	1100	1100	tr	tr
20	n-Nonanal	1103	1101	0.1	-
21	5-Methyl undecane	1156	1159	0.9	tr
22	2-Nonenal(*E*)	1163	1162	1.4	-
23	3-Methyl undecane	1170	1166	0.9	tr
24	1-Nonanol	1172	1169	0.8	-
25	Naphthalene	1182	1181	tr	-
26	1-Hexyl-3-methyl cyclopentane	1190	1189	2.3	tr
27	n-Dodecane	1200	1200	0.3	0.4
28	Dihydrocarvone	1202	1201	tr	-
29	Decanal	1203	1202	0.4	tr
30	2,4-Nonadienal	1215	1212	0.5	-
31	Carvone	1246	1243	2.0	-
32	2-Decenal(*E*)	1263	1264	1.1	-
33	1-Decanol	1274	1270	tr	-
34	2,4-Decadienal(*E,Z*)	1293	1293	0.6	-
35	n-Tridecane	1300	1300	0.3	tr
36	Carvacrol	1305	1299	0.3	-
37	2,4-Decadienal(*E,E*)	1317	1317	2.2	-
38	5-Methyl tridecane	1352	1355	0.5	tr
39	3-Methyl tridecane	1369	1369	0.5	-
40	1-Tetradecene	1392	1389	0.1	tr
41	n-Tetradecane	1400	1400	0.4	1.5
42	6-Tetradecene	1403	1405	tr	-
43	6,10-Dimethyl-2-undecanone	1405	1400	0.2	-
44	β-Ionone	1484	1489	tr	tr
45	n-Pentadecane	1500	1500	0.3	tr
46	Myristicin	1519	1519	tr	-
47	1-Hexadecene	1589	1590	-	tr
48	n-Hexadecane	1600	1600	0.5	1.2
49	Dillapiole	1622	1621	0.5	-
50	n-Heptadecane	1700	1700	0.2	tr
51	Tetradecanoic acid(Myristic acid)	1768	1773	0.1	-
52	n-Octadecane	1800	1800	1.0	1.7
53	6,10,14-trimethyl-2-pentadecanone	1842	1849	0.5	tr
54	n-Nonadecane	1900	1900	tr	tr
55	Hexadecanoic acid(Palmitic acid)	1979	1975	4.9	16.7
56	n-Eicosane	2000	2000	0.5	tr
57	n-Heneicosane	2100	2100	0.4	tr
58	9,12-Octadecadienoic acid(*Z,Z*) {Linoleic acid}	2153	2143	38.8	37.7
59	9-Octadecenoic acid(*Z*)				

Table 1. Continued

	{Oleic acid}	2158	2143	17.9	19.6
60	Octadecanoic acid(Stearic acid)	2175	2164	0.4	0.6
61	n-Docosane	2200	2200	0.2	-
62	n-Tricosane	2300	2300	tr	-
	Total identified constituents			96.1	91.8
	Fatty acid components			62.1	74.6
	Saturated fatty acids			5.4	17.3
	Unsaturated fatty acids			56.7	57.3
	Other voltiles (cyclic derivatives, aldehydes, ketones, alcohols, terpenoids, …*etc*.)			34	17.2

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 6-14; d: tr: trace (< 0.1%)

Aldehydes, ketones, alcohols (mostly with aliphatic hydrocarbon side chains), and 2-*n*-pentyl furan were detected in one or both examined oils. Xu *et al.*, reported the detection of *n*-hexanal, *n*-hexanol, and trans-2-octen-1-ol and 2-*n*-pentyl furan as the dominant compounds of seed oil of *Lactuca sativa* L. growing in Xinjiang [16]. It is proposed that 2-*n*-pentyl furan is formed by autoxidation of linoleic acid [17].

The terpenoids including α-phellandrene, limonene, dihydrocarvone, carvone, carvacrol, myristicin and dill apiole were detected in low or mostly trace amounts in the seeds oil only; while β-ionone could be detected in trace amounts in both seeds and fresh leaves oils. Al Nomaani, *et al*. reported the occurrence of limonene and nine other terpenoids (namely, α-pinene, γ-cymene, thymol, durenol, α-terpinene, thymol acetate, caryophyllene, spathulenol and camphene) as the major chemical compounds of essential oils isolated from fresh and dry leaves of *Lactuca sativa* L. native to Sultanate of Oman [18]. However, Elsharkawy *et al.* who analyzed the essential oil of fresh leaves of a wild *Lactuca* species (*Lactuca serriolla* L.) growing under dry desert condition of Northern Region in Saudi Arabia [19], also reported the occurrence of limonene and five other terpenoids (α-pinene, germacrene D, trans-β-caryophyllene, caryophyllene oxide and santolina triene) as the major constituents.

In conclusion, our results showed that both essential oils obtained from the seeds and fresh leaves of the plant grown in Isfahan mainly composed of a mixture of saturated and unsaturated fatty acids.

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Author contributions

Suleiman Afsharypuor, Mohammad Mazaheri, and Fereshteh Shakibaei were supervisors of the Ph.D thesis; Abolfazl Aslani was the adviser of the Ph.D thesis; Mahdieh Ranjbar was the Ph.D student who carried out the thesis research.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

GC: Gas Chromatography; GC/MS: Gas Chromatography/Mass Spectroscopy; μA: Micro Ampere; RI: Retention Index; tr: Trace