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Kelussia odoratissima Mozaff. a rich source of essential fatty acids and phthalides

Mehdi Ghasemi[1], Aghafakhr Mirlohi[2*], Mahdi Ayyari[1], Abdolali Shojaeiyan[1]

[1]Department of Horticulture Science, Tarbiat Modares University, Tehran, Iran
[2]Department of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran

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ABSTRACT

Introduction: The present study was aimed to assess the fatty acids of leaf and essential oil compositions of of aerial parts of *Kelussia odoratissima*.

Methods: The aerial parts of *K. odoratissima* from the three habitats were dried. The essential oils were obtained by hydrodistillation for 3 hours in a Clevenger-type apparatus, then the analysis of the components was carried out using gas chromatography–mass spectrometry (GC-MS). To study the oil yield and fatty acids, the dried leaves were subjected to extraction in hexane by using Soxhlet apparatus. To analyze fatty acids from the oil fractions by GC technique, the oil was subjected to transesterification to obtain the fatty acid methyl esters (FAMEs), which were dissolved in hexane and were subjected to GC analysis.

Results: According to the results, a total of 43 components were detected, the major constitutes of essential oil compositions were (*Z*)-Ligustilide (76.45%), Unknown-A (4.47%), (*E*)-Ligustilide (2.57%), (*Z*)-Butylidene phthalide (2.37%), 5-pentyl cyclohexa-1,3-diene (1.57%) and kessane (0.77%). Sixteen fatty acids were separated from the oil (5% yield per 100 g dry matter). Linoleic acid (25.46%), α-linolenic acid (16.66%), palmitic acid (11.92%), oleic acid (9.33%), stearic acid (4.72%), petroselinic acid (2.53%), arachidonic acid (2.51%) and erucic acid (1.76%) were the major fatty acids.

Conclusion: Generally, *K. odoratissima* is a rich source of essential fatty acids and phthalide derivatives, specially (*Z*)-ligustilide. This study presented valuable information about the phytochemical properties, which can be useful for the future research on the pharmacological effects of *K. odoratissima*.

Implication for health policy/practice/research/medical education:
Kelussia odoratissima with pharmaceutical beneficial compounds such as (*Z*)-Ligustilide, ω-3 and ω-6 can be served as fresh and garnish in the diet. Reducing high blood pressure and cardiovascular diseases by these components has been proved in other researches.

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Introduction

Ethnopharmacology is a scientific study on the biological activities of botanical drugs used by local people, which have beneficial, toxic or other direct pharmacological effects (1). Iran has appreciable past regarding folk medicines, especially based on medicinal plants because its flora includes about 8000 plant species and endemic species (2).

Kelussia odoratissima Mozaff. is a wild, perennial and medicinal herb that belongs to the Apiaceae family. This species is found only in Central Zagros Mountains, and so far there has not been reported in other areas of the world (3). The aerial parts harvested and sold in the local markets in the early spring as spice and has been used to cure inflamed ulcers, hypertension, and cardiovascular diseases by the indigenous people (4). The genetic and ecological factors such as precipitation, temperature, plant competition and edaphic conditions, affects the chemical variation of the medicinal plants (5). The results of the study by Shojaei et al showed that the dominant consti-

***Corresponding author**: Aghafakhr Mirlohi, Department of Agronomy and Plant Breeding, Isfahan University of Technology, Isfahan, Iran. Tel: +98 31 33913450, Fax: +98 31 33913324, Email: : mirlohi@cc.iut.ac.ir

tutes of essential oil of *K. odoratissima* are phthalides from the Koohrang, Bazoft and Samsami habitats in Iran (6). In the other studies, phthalides such as (*Z*)-Ligustilide, (*E*)-Ligustilide, (*Z*)-Butyldiene phthalide, (*E*)-Butyldiene phthalide and 3-*n*-Butylphthalide have been reported as the major compositions of essential oil (7,8).

Edible oils are biological mixtures of plant origin consisting of ester mixtures derived from glycerol with chain of fatty acids, which play a crucial role in growth and development of the body (9). Fatty acids are usually classified in classes as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA). On the other hand, the unsaturated fatty acids are classified into a series known as omega, being omega-3, omega-6, omega-9 and Omega-12 as the essential fatty acids (EFAs) (10). In a related study, only the fatty acids of the seeds of *K. odoratissima* were discussed (11).

In Iran, most of the researches on *K. odoratissima* have been focused on the limited and unknown habitats. To the best of our knowledge, there is no report on the fatty acids of aerial parts and the chemical compounds of *K. odoratissima* from the major habitats of Dareh-Sepestan, Landeroun (Isfahan province) and Vastegan (Chaharmahal and Bakhtiari province). The aim of this study was to investigate the essential oil compositions and the fatty acids of aerial parts of *K. odoratissima* using gas chromatography-mass spectrometry (GC-MS) from the above mentioned habitats.

Materials and Methods

Essential oil extraction and analysis

The aerial parts (leaves) of *K. odoratissima* from the habitats of Dareh-Sepestan, Vastegan (Isfahan province) and Landeroun (Chaharmahal and Bakhtiari province), Iran, were dried at room temperature in shade at 25 ± 3°C. The essential oil of samples was obtained by hydrodistillation for 3 hours in a Clevenger-type apparatus according to the method recommended (12). The essential oils were dried over anhydrous Na_2SO_4, and stored at 4°C before analysis. GC analysis was carried out using a Thermoquest gas chromatograph with a flame ionization detector (FID) equipped with a fused silica capillary DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Detector (FID) temperature was 280°C and the injector temperature was 250°C. Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min within a split ratio of 1:100; the oven temperature program was 60°C–250°C at the rate of 5°C min^{-1} and finally held isothermally for 10 minutes. GC–MS analysis was carried out with a Thermoquest–Finnigan gas chromatograph equipped with fused silica capillary DB-5 column (60 m × 0.25 mm i.d., 0.25 µm film thickness) coupled with a TRACE mass spectrometer (Manchester, UK). Helium was used as the carrier gas with an ionization energy of 70 eV, the mass range was 40–460 a.m.u. Ion source, and interface temperatures were 200°C and 250°C, respectively. The oven temperature program was similar as above for the GC. Compositions were identified based on their retention indices (*n*-alkanes), by comparison of their mass spectra with those reported in the literature and stored in NIST, Willey and Adams libraries.

Fatty acid extraction and analysis

The plant sample was collected from the Birahgan habitat in Chaharmal and Bakhtiari province, weighed and dried to remove excess moisture. The oil was extracted for 5 hours using hexane as the extraction solvent. The extract was then evaporated, dried and weighed.

To prepare fatty acid methyl esters (FAMEs), 2% NaOH and 2 mg pentadecanoic acid (C15:0) as an internal standard were added to the 0.11 g oil, which was incubated for 10 minutes in a heated bath and cooled. The methanolic BF_3 (2.18 ml) was then added, and the sample was heated in the bath for 2 to 3 minutes and cooled again. In the following, FAMEs was dissolved in 1.5 ml hexane, and partitioned in 1 ml of saturated sodium chloride and then vigorously shacked. In the final step of dehydration, 1 ml of upper layer was separated and was mixed with 0.5 mg sodium sulfate by centrifuging. The upper layer was injected to GC.

The identification of FAMEs was carried out by GC (UNICAM 4600, SB Analytical, UK) equipped with GC-FID and a BPX-70 fused-silica capillary column (30 m length, 0.25 mm i.d., 0.22 µm film thickness). The operating conditions were as follows: the initial temperature was 50°C, increased by 5°C min^{-1} up to the 160°C; and with a ramp of 20°C min^{-1} reached 180°C and finally 200°C. The injector and detector temperatures were 250 and 250°C, respectively. The column head pressure (Helium) was 20 psi. Each fatty acid was identified in its methyl ester form by comparing its retention time with the internal standard.

Results

Chemical compositions of essential oils

The percentages and retention indices of the compounds in the essential oils of *K. odoratissima* accessions are listed in Table 1. In total, the number of compounds identified and quantified were 38 in Dareh-Sepestan, 42 in Vastegan and 39 in Landeroun accessions, representing 97.90%, 98.13% and 95.65% of the total oil, respectively.

In the essential oil of Dareh-Sepestan accession, (*Z*)-Ligustilide (76.09%), Unknown-A (7.40%), 5-pentyl cyclohexa-1,3-diene (2.21%), (*E*)-Ligustilide (2.16%), (*Z*)-Butylidene phthalide (1.40%), kessane (1.07%) and α-copaene (0.81%) were the main constituents. (Z)-Ligustilide (75.87%), unknown-A (3.93%), (*E*)-Ligustilide (3.20%), (Z)-Butylidene phthalide (2.47%), 5-pentyl cyclohexa-1,3-diene (2.15%), α-copaene (0.93%) and (*Z*)-γ-Bisabolene (0.89%) were the main constituents in the essential oil of Vastegan accession. In the essential oil of Landeroun accession, (Z)-Ligustilide (77.40%), (Z)-Butylidene phthalide (3.25%), unknown-A (2.39%), (*E*)-Ligustilide (2.35%), (Z)-β-Santalol acetate (1.03%), 5-pentyl cyclohexa-1,3-diene (0.89%), 4-Hydroxybenzaldehyde (0.84%) were the main constituents.

Table 1. Chemical composition of essential oils (%) of three accessions of *K. odoratissima*

No.	Component	RI[a]	RI[b]	1[c]	2	3	Mean
1	Propyl benzene	951	951	0.19	0.09	0.13	0.14
2	(*E*)-2-methyl-3-Octen-5-yne	953	958	0.32	0.10	0.06	0.16
3	Limonene	1027	1024	0.17	0.09	0.10	0.12
4	α-Terpinolene	1088	1086	0.28	0.16	0.16	0.20
5	5-Pentyl cyclohexa-1,3-diene	1159	1156	2.21	2.15	0.89	1.75
6	Unknown-A	1277	-	7.40	3.93	2.39	4.57
8	2-Undecanone	1291	1293	-	0.12	-	0.12
9	Menthyl acetate	1294	1294	0.17	-	0.07	0.12
10	p-Vinylguaiacol	1315	1315	0.19	0.46	0.12	0.26
11	α-Cubebene	1351	1351	0.08	0.13	0.08	0.10
12	4-Hydroxybenzaldehyde	1354	1355	0.17	0.39	0.84	0.47
13	α-Copaene	1377	1377	0.81	0.93	0.06	0.60
14	9-Decenyl acetate	1389	1384	0.06	0.14	0.09	0.10
15	(*Z*)-Caryophyllene	1406	1406	0.23	0.18	0.19	0.20
16	(*E*)-Caryophyllene	1421	1421	0.25	0.34	0.22	0.27
17	α-Humulene	1455	1455	0.31	0.42	0.28	0.34
18	α-Acoradiene	1466	1465	0.48	0.34	0.37	0.40
19	10-*epi*-β-acoradiene	1475	1475	0.42	0.61	0.24	0.42
20	Germacrene D	1481	1481	0.31	0.52	0.40	0.41
21	(*Z,E*)-α-Farnesene	1489	1489	0.12	0.17	0.13	0.14
22	(E)-Muurola-4(14),5-diene	1493	1493	0.07	0.19	0.06	0.11
23	β-Himachalene	1499	1499	0.46	0.18	0.35	0.33
24	β-Bisabolene	1505	1505	-	0.21	-	0.21
25	β-Curcumene	1508	1508	0.13	0.23	0.22	0.19
26	(*Z*)-γ-Bisabolene	1513	1514	0.48	0.89	0.31	0.56
27	δ-Cadinene	1522	1522	0.13	0.66	0.68	0.49
28	Kessane	1528	1529	1.07	0.47	0.78	0.77
29	γ-Cuprenene	1531	1532	0.19	0.13	0.24	0.19
30	β-Spathulenol	1579	1577	-	0.14	0.08	0.11
31	3-Butylhexahydro phthalide	1638	1632	0.06	0.06	-	0.06
32	3-*n*-Butylphthalide	1654	1656	0.11	0.38	0.40	0.30
33	(*Z*)-Butylidene phthalide	1676	1677	1.40	2.47	3.25	2.37
34	(*E*)-Butylidene phthalide	1722	1717	0.15	0.42	0.32	0.30
35	(*Z*)-Ligustilide	1759	1741	76.09	75.87	77.40	76.45
36	(*E*)-Ligustilide	1805	1797	2.16	3.20	2.35	2.57
37	(*Z*)-β-Santalol acetate	1823	1818	0.35	0.59	1.03	0.66
38	Neophytadiene	1834	1841	0.05	0.07	0.10	0.07
39	*Z*-Ternine [d]	1848	1845	-	0.06	0.08	0.07
40	*n*-Nonadecane	1894	1900	0.19	0.06	-	0.13
41	Hexadecanoic acid	1958	1959	0.10	0.17	0.27	0.18
43	Phytol	2114	2114	0.06	0.09	0.34	0.16
	Total			97.42	97.81	95.08	

According to the average percentage of chemical compositions, the major constitutes were (*Z*)-Ligustilide, unknown-A, (*E*)-Ligustilide, (*Z*)-Butylidene phthalide, 5-pentyl cyclohexa-1,3-diene and kessane in all the essential oils.

The classification of the identified compounds, based on chemical groups, is shown in Figure 1. The principle compounds in the essential oil of the three accessions of *K. odoratissima* were phthalides (82.12%), followed by 'others' (8.91%), sesquiterpene hydrocarbons (5.96%) and monoterpene hydrocarbons and oxygenated sesquiterpenes were very low in the percentage. Unknown components and hydrocarbons were classified into 'others' group.

Fatty acid composition

The oil yield of the leaf of *K. odoratissima* was 5% (per 100 g dry matter). Table 2 shows the fatty acid compositions of *K. odoratissima* leaf as mg/100 g and percentage. The number of fatty acids identified were fifteen. In the oil, obtained from leaf, Linoleic acid (25.46%), α-linolenic acid

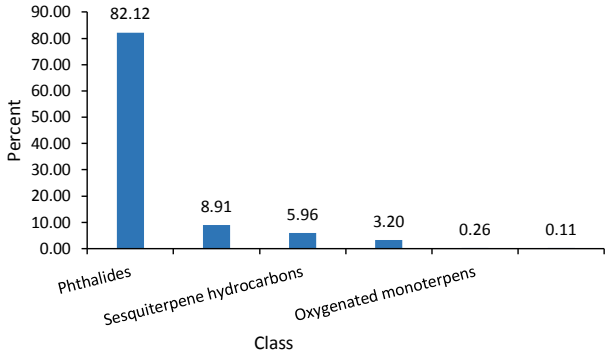

Figure 1. The classification of essential oil compositions of *K. odoratissima*, based on chemical groups.

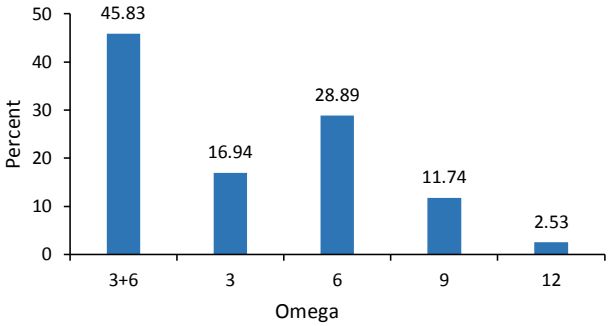

Figure 2. The classification of fatty acids of *K. odoratissima*, based on the omega type.

Table 2. Fatty acid compositions of *K. odoratissima* Mozaff. aerial parts

No.	Fatty acid	mg/100 g	%	Structure
1	Palmitic acid	61.74	11.92	C16:0
2	Palmitoleic acid	3.34	0.64	C16:1n7
3	Heptadecanoic acid	0.18	0.03	C17:0
4	Stearic acid	24.48	4.72	C18:0
5	Petroselinic acid	13.11	2.53	C18:1n12
6	Oleic acid	48.33	9.33	C18:1n9
7	Linoleic acid	131.92	25.46	C18:2n6c
8	Linolelaidic acid	3.13	0.60	C18:2n6t
9	α-Linolenic acid	86.33	16.66	C18:3n3
10	Arachidic acid	5.17	0.10	C20:0
11	Eicosenoic acid	3.38	0.65	C20:1n9
12	Eicosapentaenoic acid	1.45	0.28	C20:5n3
13	Henicosanoic acid	1.64	0.32	C21:0
14	Arachidonic acid	12.99	2.51	C20:4n6
15	Erucic acid	9.14	1.76	C22:1n9

(16.66%), palmitic acid (11.92%), oleic acid (9.33%), stearic acid (4.72%), petroselinic acid (2.53%), arachidonic acid (2.51%) and erucic acid (1.76%) were the major fatty acids. The presence of other fatty acids was very low. Fatty acids were classified in classes as SFA, MUFA and PUFA. The percentage of PUFA, MUFA and SFA was 45.83, 14.91 and 23.17, respectively.

The fatty acids identified from *K. odoratissima* oil were categorized based on the type of omega (Figure 2). Omega-6 had the highest percentage (28.89%). The total of EFAs including omega-3 and omega-6 was 45.83%.

Discussion

Based on our findings, (*Z*)-Ligustilide is the dominant component in the essential oil of *K. odoratissima*, which is in accordance with previous reports (7,8,13). The other components such as (*Z*)-Butylidene phthalides, (*E*)-Ligustilide, Kessane, 3-*n*-Butylphthalide and caryophyllene oxide reported as the main compounds of essential oil of *K. odoratissima* (6). (*Z*)-Ligustilide, (*Z*)-Butylidene phthalide, unknown, 5-Pentyl cyclohexa-1,3-diene, kessane, cuparene, β-cadinene and pulegone have been identified as the main components (14). The second main compound (2.39 to 10.34%) was not fully identified and it has also been reported by the other researchers, with a similar retention index, as an unknown compound (14,15).

Phthalides are rich in the essential oil of *K. odoratissima*. The anti-inflammatory effects of ligustilides from *Ligusticum chuanxiong* and *Angelica sinensis* have been confirmed (16,17). The studies indicate that (*Z*)-ligustilide can facilitate blood circulation, attenuate pain behavior in mice, act to limit ischemic brain damage in rats and exhibit the strongest antioxidant activity (18,19). Some of the studies have also focused on relaxation of smooth muscles in the gastrointestinal, respiratory and circulatory systems and hepatoprotective, immunomodulatory and cardiovascular properties (20). 3-Butylhexahydro phthalide and (*Z*)-Ternine, as known phthalide derivatives are reported for the first time from *K. odoratissima*, but have been reported from *Levisticum officinale* (21).

Petroselinic acid was one of fatty acids in the oil of *K. odoratissima.* The presence of Petroselinic acid (72.35%) in the seeds of *K. odoratissima* was also reported by Saaidi and Omidbaigi (11). It is an uncommon and rare fatty acid that comprises nearly 85% of the total fatty acids of Apiaceae seeds (22). This fatty acid is used to show chemotaxonomical relationships among the genera of the Apiaceae family (23). Petroselinic acid as an unsaturated fatty acid and isomers of oleic acid with a melting point higher than oleic acid can be useful in cosmetics, pharmaceuticals, and perfume and food industries (24).

According to the percentage of standard fatty acids in dietary fats (25,26), the total saturated fatty acids in *K. odoratissima* leaf represent lower value of 23.17% in comparison with the coconut, palm and cottonseed oils, while being higher than the other oils. In comparison with fatty acids in dietary fats, the percentage of MUFAs (14.91%) was more than all the dietary fats, except in coconut and safflower oils. Foods containing MUFAs reduce low-density lipoprotein (LDL) cholesterol, while possibly increasing high-density lipoprotein (HDL) cholesterol (27). PUFAs are also present in much more amounts than coconut, palm, olive and rapeseed oils. PUFAs protect against car-

diovascular disease by providing more membrane fluidity than MUFAs, but they are more vulnerable to lipid peroxidation (28). EFAs in *K. odoratissima* leaf have higher value of 45.53% in comparison with the coconut, palm, olive, sunflower and rapeseed oils, while being lower than the cottonseed, soya, corn and safflower oils. Both omega-3 and omega-6 are important components of cell membranes and these needed for growth and repair. The human body is not capable of producing them. The plants and seed oils are very good sources to obtain the essential fatty acids (29,30).

In summary, this study investigated the fatty acid profile of the leaf and the chemical compositions of new three accessions of *K. odoratissima*. Overall, *K. odoratissima* is a rich source of essential fatty acids and phthalide derivatives, especially (*Z*)-ligustilide with the effects of diverse biological activities. This study provided valuable information about the phytochemical properties, which can be useful for the subsequent studies on the pharmacological effects of *K. odoratissima.*

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Authors' contributions

MGh did the experiment and prepared the first draft. All authors contributed in the conception and design of the work, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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