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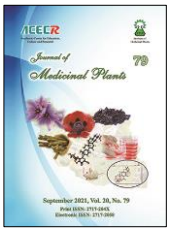

Research Article

Phytochemical constituents of the fruits of *Kelussia odoratissima* Mozaff., an aromatic plant endemic to Iran

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ABSTRACT

Background: *Kelussia odoratissima* Mozaff. from Apiaceae family is a perennial herbaceous plant endemic to the west of Iran. The aromatic aerial parts of this species are traditionally used by indigenous people to flavor some local foods, as well as for various therapeutic purposes. **Objective:** The present study was designed to analyze phenolic compounds and essential oil constituents of *K. odoratissima* fruits. **Methods:** The *n*-butanol fraction obtained from hydroalcoholic extract of *K. odoratissima* fruits was investigated by chromatography on normal phase and Sephadex LH-20 columns. Chemical structures of the isolated compounds were clarified by ^{1}H-NMR and ^{13}C-NMR spectral analyses. Essential oil constituents of the fruits were also analyzed using GC-MS. **Results:** Phytochemical investigation of the *K. odoratissima* fruits resulted in the isolation of five flavonol glycosides; isorhamnetin 3-O-glucoside (**1**), quercetin 3-O-glucoside (isoquercetin) (**2**), isorhamnetin 3-O-rutinoside (narcissin) (**3**), isorhamnetin 3-O-glucuronide (**4**) and quercetin 3-O-glucuronide (mequilianin) (**5**). GC-MS analysis of the fruits essential oil led to the identification of the thirty six compounds, of which (*Z*)-ligustilide (15.93 %), *δ*-cadinene (12.26 %) and germacrene D (12.18 %) were the main compounds. **Conclusion:** The results of this study introduce *K. odoratissima* fruits as a source of flavonoid glycosides and phthalate derivatives. The presence of these compounds with known biological properties and health beneficial effects provides more medicinal potentials for the fruits of *K. odoratissima* and suggest it an appropriate option for further studies.

1. Introduction

The genus *Kelussia* from Apiaceae family is represented by only one member, *Kelussia odoratissima* Mozaff., which is found in central Zagros mountains, west of Iran [1]. The aerial parts of this aromatic species are used by indigenous people under the local names of "Kellos" and "Karafse-Bakhtiari" as vegetable, as flavoring agent in yogurt, for the treatment of indigestion, rheumatism, gastric ulcer, cough,

Abbreviations: NMR, Nuclear magnetic resonance; GC-MS, Gas Chromatography-Mass Spectrometry; TLC, Thin Layer Chromatography

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pain, diabetes and as a sedative agent [2, 3]. A review on literature shows that extracts and essential oils obtained from different parts of *K. odoratissima* have been reported for their biological and pharmacological activities such as antioxidant [4, 5], antibacterial [6], larvicidal [7], antileishmanial [8], cytotoxic [9], spasmolytic [10], anti-inflammatory [11], antihypertensive [12], sedative and anxiolytic [13] effects. One study reported the isolation of two steroids, stigmasterol and *β*-sitosterol and one phthalide, 3-butyliden-4,5-dihydrophthalide from the *n*-hexane extract of *K. odoratissima* fruits [14]. Furthermore, they identified thirty eight compounds in the fruits essential oil, of which (*Z*)-ligustilide (29.2 %), germacrene B (15.9 %) and germacrene D (15.5 %) were the major compounds [14]. Another study reported the analysis of fatty acids in the fruits of *K. odoratissima* and showed that among the five fatty acids identified in the fruits oil, petroselinic acid (72.35 %) and linoleic acid (19.14 %) were the main acids [15]. In the mentioned study, *K. odoratissima* fruits were also reported as a source of phenolic compounds by a total phenolic content of 218.15 milligrams gallic acid equivalents (GAE) per gram of dry fruit weight [15]. Beside the high phenolic content, there is not any report on phenolic principles of *K. odoratissima* fruits. Therefore, the present study was designed to isolation and structural elucidation of the phenolic compounds present in the fruits of this valuable medicinal plant. The essential oil composition of the fruits was also identified and compared by related data previously reported for *K. odoratissima*.

2. Materials and Methods

2.1. Plant material

The fruits of *Kelussia odoratissima* Mozaff. were purchased from Pakan-Bazr Co., Isfahan, Iran (Plant source: Fereydunshahr region, Isfahan, Iran.; Collection date: July 2017). The identity of the fruits was authenticated by botanist Dr. Yousef Ajani (Research Institute of Forest and Rangelands, Tehran, Iran) and the code of PMP-2694 was assigned for it at the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

2.2. Extraction of Phenolics

The dried and comminuted fruits of *K. odoratissima* (1.4 kg) were subjected to extraction using maceration method with dichloromethane and methanol-water (70:30), successively (4 × 5 L, each). The obtained extracts were concentrated using a rotary evaporator at 45 °C under the reduced pressure and then dried in vacuum oven. Hydroalcoholic extract (250 g) was suspended in water (0.5 L) and fractionated by equal volume of *n*-butanol (×3) to extract its phenolic compounds.

2.3. Isolation and purification of the compounds

A portion of the *n*-butanol fraction (20 g) was chromatographed on a normal phase silica gel column (Mesh 230-400, Merck) using a gradient solvent mixture of $CHCl_3$-MeOH (100:0 to 50:50) to get eighteen subfractions (B1-B18). Thin layer chromatography (Pre-coated silica gel GF_{254} plates, Merck) was applied to monitor column chromatography and fractions giving similar spots under UV (254 and 366 nm) followed by the exposure to ammonia vapor were combined. Based on the TLC analysis, subfractions B6, B10, B11, B14 and B16 were chosen for further isolation. Column chromatography of these subfractions (B6, B10, B11, B14 and B16) on a Sephadex LH-20 column using methanol as mobile phase resulted in the isolation of compounds **1** (19.0 mg), **2**

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(13.5 mg), **3** (35.4 mg), **4** (23.8 mg) and **5** (18.1), respectively. The structures of the compounds were elucidated by ^{1}H-NMR and ^{13}C-NMR (Varian- INOVA, 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) spectral analysis.

2.4. Essential oils extraction

The comminuted fruits (100 g) were subjected to hydrodistillation for 3 hours, using a Clevenger apparatus. The obtained oils were dried over anhydrous sodium sulfate and keep at 4 °C until analysis.

2.5. GC-MS analysis

The essential oil extracted from the fruits of *K. odoratissima* were analyzed on a HP-6890 gas chromatograph with a BPX5 column (30 m × 0.25 mm id, 0.25μm film thickness), equipped with HP-5973 mass detector (Ionization energy: 70 eV) under the following conditions; 5 min after injection, oven temperature was increased from 50 °C to 240 °C at a rate of 3 °C/min and then reached to 300 °C at the rate of 15 °C/min and hold 3 min in this temperature. Injector temperature: 250 °C, detector temperature: 220 °C, injection volume: 1.0 μl, split ratio: 1:35, carrier gas: helium (99.999 %, Flow rate: 0.5 ml/min). The retention indices (RIs) were calculated for all identified compounds using a homologous series of *n*-alkanes (C_8-C_{24}) injected under the same conditions described for the analyzed essential oil. Identification of the compounds were carried out based on computer matching with the Wiley 275.L library, as well as by comparison of RIs and mass fragmentation patterns with those published for standard compounds [16].

3. Results

3.1. Isolation and structural elucidation

Phytochemical analysis of the fruits of *K. odoratissima* using chromatography on normal phase silica gel and Sephadex LH-20 columns resulted in the isolation of five compounds (**1**-**5**) from *n*-butanol fraction of hydroalcoholic extract. The structures of the isolated compounds were characterized as isorhamnetin 3-O-*β*-D-glucopyranoside (**1**), quercetin 3-O-*β*-D-glucopyranoside (isoquercetin) (**2**), isorhamnetin 3-O-β-D-glucopyranosyl-(6→1)-*α*-L-rhamnopyranside (isorhamnetin 3-O-*β*-D-rutinoside, narcissin) (**3**), isorhamnetin 3-O-*β*-D-glucuronide (**4**) and quercetin 3-O-*β*-D-glucuronide (mequilianin) (**5**) (Fig. 1) using ^{1}H-NMR and ^{13}C-NMR spectral analyses, as well as by comparison with published data [17-21].

3.1.1. Spectroscopic data of the isolated compounds

Compound **1**; *Isorhamnetin 3-O-β-D-glucopyranoside* ($C_{22}H_{22}O_{12}$); Yellow amorphous solid; R_f = 0.6 ($CHCl_3$-MeOH, 8:2); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 7.93 (1H, *d*, *J* = 2.0 Hz , H2′), 7.50 (1H, *dd*, *J* = 8.5 and 2.0 Hz, H6′), 6.93 (1H, *d*, *J* = 8.5 Hz, H5′), 6.46 (1H, *d*, *J* = 2.0 Hz , H8), 6.22 (1H, *d*, *J* = 2.0 Hz , H6), 5.56 (1H, *d*, *J* = 7.4 Hz, H1″), 3.83 (3H, *s*, OCH_3), 3.1-3.7 (6H, *overlapped signals*, H2″-H6″). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ_C 177.78 (C4), 164.55 (C7), 161.35 (C5), 156.87 (C9),156.73 (C2), 149.63 (C3′), 147.28 (C4′), 133.46 (C3), 122.51 (C6′), 121.58 (C1′), 115.57 (C5′), 113.87 (C2′), 104.43 (C10) , 101.18 (C1″), 99.10 (C6), 94.22 (C8), 77.76 (C5″), 76.62 (C3″), 74.63 (C2″), 70.12 (C4″) , 60.93 (C6″), 56.15 (OCH_3) [17].

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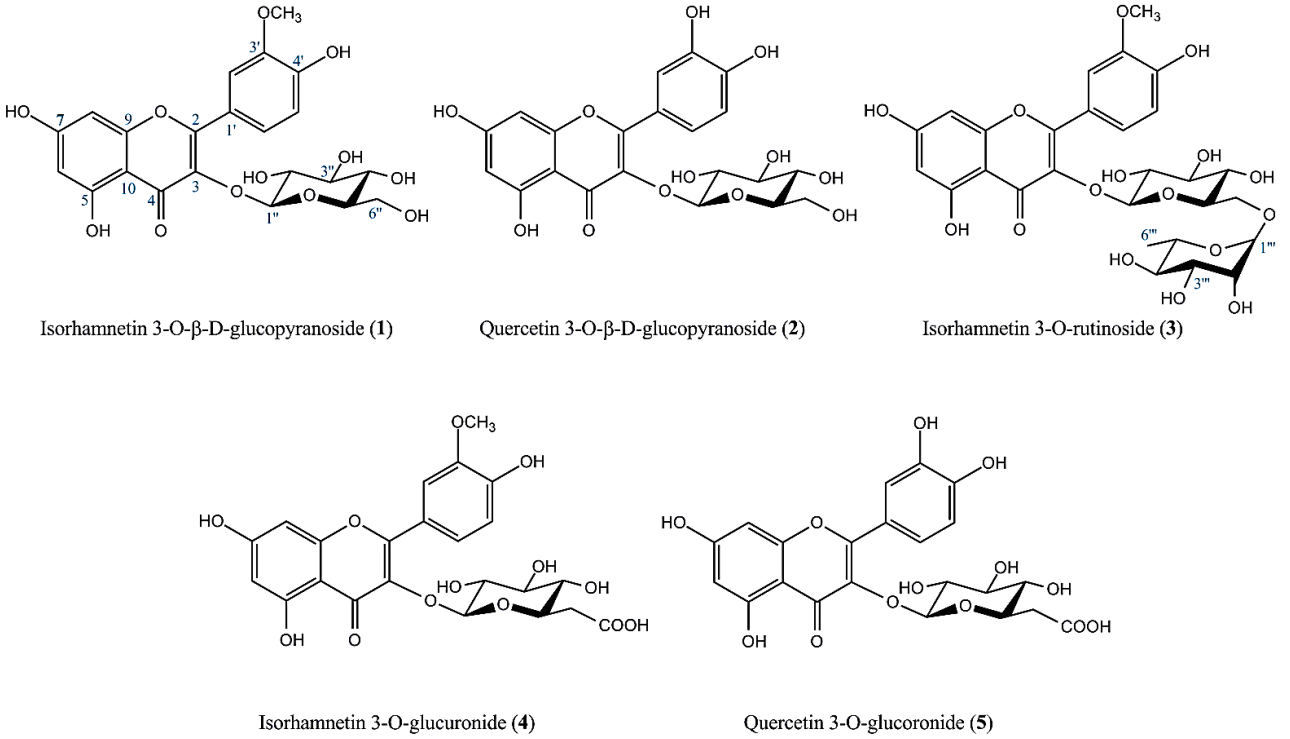

Fig. 1. The structures of isolated compounds (**1**-**5**) from *K. odoratissima* fruits

Compound **2**; *Quercetin 3-O-β-D-glucopyranoside* (*isoquercetin*) ($C_{21}H_{20}O_{12}$); Yellow amorphous solid; R_f = 0.5 ($CHCl_3$-MeOH, 8:2); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 7.58 (1H, *d*, *J* = 2.0 Hz, H2′), 7.56 (1H, *dd*, *J* = 9.0 and 2.0 Hz, H6′), 6.86 (1H, *d*, *J* = 9.0 Hz, H5′), 6.42 (1H, *d*, *J* = 2.0 Hz, H8), 6.21 (1H, *d*, *J* = 2.0 Hz, H6), 5.45 (1H, *d*, *J* = 7.5 Hz, H1″), 3.1-3.7 (6H, *overlapped signals*, H2″-H6″). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ_C 177.82 (C4), 164.52 (C7), 161.37 (C5), 156.90 (C9),156.60 (C2), 148.69 (C4′), 145.07 (C3′), 133.80 (C3), 122.09 (C6′), 121.63 (C1′), 116.55 (C5′), 115.60 (C2′), 104.36 (C10), 101.29 (C1″), 99.05 (C6), 94.02 (C8), 77.88 (C5″), 76.73 (C3″), 74.42 (C2″), 70.21 (C4″) , 61.26 (C6″) [18].

Compound **3**; *Isorhamnetin 3-O-β-D-rutinoside* (*narcissin*) ($C_{28}H_{32}O_{16}$); Yellow amorphous solid; R_f = 0.3 ($CHCl_3$-MeOH, 8:2); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 7.83 (1H, *d*, *J* = 1.8 Hz, H2′), 7.51 (1H, *dd*, *J* = 8.4 and 1.8 Hz, H6′), 6.91 (1H, *d*, *J* = 8.4 Hz, H5′), 6.44 (1H, *d*, *J* = 2.0 Hz, H8), 6.21 (1H, *d*, *J* = 2.0 Hz, H6), 5.40 (1H, *d*, *J* = 7.4 Hz, H1″), 4.40 (1H, *br s*, H1‴), 3.83 (3H, *s*, OCH_3), 3.0-3.7 (10H, *overlapped signals*, H2″-H6″ and H2‴-H-5‴), 0.94 (3H, *d*, *J* = 6.2 Hz, H6‴); ^{13}C-NMR (DMSO-d_6, 125 MHz): δ_C 177.71 (C4), 164.34 (C7), 161.26 (C5), 157.01 (C9), 156.94 (C2), 149.57 (C4′), 147.26 (C3′), 133.48 (C3), 122.77 (C6′), 121.54 (C1′), 115.57 (C5′), 113.63 (C2′), 104.44 (C10), 101.54 (C1″), 101.25 (C1‴), 99.08 (C6), 94.31 (C8), 76.54 (C5″), 76.20 (C3″), 74.52 (C2″), 72.03 (C4‴), 70.82 (C3‴), 70.57 (C2‴), 70.39 (C4″), 68.68 (C5‴), 67.37 (C6″), 56.12 (OCH_3), 17.99 (C6‴) [19].

Compound **4**; *Isorhamnetin 3-O-β-D-glucuronide* ($C_{22}H_{20}O_{13}$); Yellow amorphous solid; R_f = 0.5 ($CHCl_3$-MeOH, 8:2); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 8.06 (1H, *br s*, H2′),

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7.36 (1H, *br d*, *J* = 8.4, H6′), 6.87 (1H, *d*, *J* = 8.4 Hz, H5′), 6.16 (1H, *br s*, H8), 5.99 (1H, *br s*, H6), 5.57 (1H, *d*, *J* = 7.7 Hz, H1″), 3.80 (3H, *s*, OCH_3), 3.21-3.39 (4H, *overlapped signals*, H2″-H5″). ^{13}C-NMR (DMSO-d_6, 125 MHz): δ_C 177.55 (C4), 173.13 (C6″), 165.44 (C7), 161.21 (C5), 156.81 (C9), 156.81 (C2), 149.38 (C4′), 147.14 (C3′), 133.23 (C3), 122.24 (C6′), 121.68 (C1′), 115.31 (C5′), 114.48 (C2′), 103.68 (C10), 101.28 (C1″), 99.02 (C6), 94.28 (C8), 76.42 (C3″), 76.61 (C5″), 74.35 (C2″), 72.44 (C4″), 56.13 (OCH_3) [20].

Compound **5**; *Quercetin 3-O-β-D-glucuronide* (*mequilianin*) ($C_{21}H_{18}O_{13}$); Yellow amorphous solid; R_f = 0.4 ($CHCl_3$-MeOH, 8:2); ^{1}H-NMR (DMSO-d_6, 500 MHz): δ_H 8.23 (1H, *br s*, H2′), 7.33 (1H, *dd*, *J* = 8.4 and 1.2 Hz, H6′), 6.83 (1H, *d*, *J* = 8.4 Hz, H5′), 6.24 (1H, *br s*, H8), 6.06 (1H, *br s*, H6), 5.15 (1H, *d*, *J* = 7.3 Hz, H1″), 3.1-3.5 (4H, *overlapped signals*, H2″-H6″).^{13}C-NMR (DMSO-d_6, 125 MHz): δ_C 177.27 (C-4), 172.95 (C6″), 165.06 (C7), 160.99 (C5), 157.25 (C9), 157.25 (C2), 148.62 (C4′), 145.04 (C3′), 134.35 (C3), 121.05 (C6), 121.05 (C1′), 118.10 (C-5′), 115.81 (C-2′), 103.52 (C10), 103.52 (C1″), 100.27 (C-6), 94.79 (C-8), 76.92 (C-3″), 74.54 (C-5″), 74.41 (C-2″), 72.05 (C-4″) [21].

3.2. Essential oil composition

Hydrodistillation of the fruits of *K. odoratissima* led to the extraction of a pale yellowish oil with the yield of 1.5 % (v/w). GC-MS analysis of the obtained essential oil resulted in the identification of the thirty six compounds, representing 90.74 % of the oil (Table 1). Among the identified compounds, (*Z*)-ligustilide (15.93 %), *δ*-cadinene (12.26 %) and germacrene D (12.18 %) were the main compounds (Fig. 2) and oxygenated sesquiterpenes with relative percentage of 53.68 % were the main group of constituents identified in essential oil of *K. odoratissima* fruits.

Table 1. Chemical composition of the essential oil of *K. odoratissima* fruits obtained by hydrodistillation method

No.	Compound name[a]	%	RI[b]
1	*α*-Pinene	0.09	939
2	*β*-Pinene	0.35	978
3	2-Ethyl-2-hexenal	0.71	981
4	*β*-Phellandrene	3.23	1027
5	5-Pentylcyclohexa-1,3-diene	1.43	1161
6	Lavandulol	0.14	1170
7	Acetic acid octyl ester	0.24	1215
8	Citronellol	0.27	1227
9	Lavandulyl acetate	0.75	1292
10	Ylangene	0.62	1374
11	*α*-Copaene	2.92	1378
12	*β*-Cubebene	0.48	1388
13	*β*-Elemene	2.04	1391
14	*α*-Barbatene	0.42	1415
15	Lavandulyl isobutyrate	3.80	1417
16	*γ*-Elemene	4.34	1435
17	Sativene	1.36	1437
18	*α*-Humulene	1.09	1453
19	*γ*-Muurolene	0.49	1481
20	Germacrene D	12.18	1483

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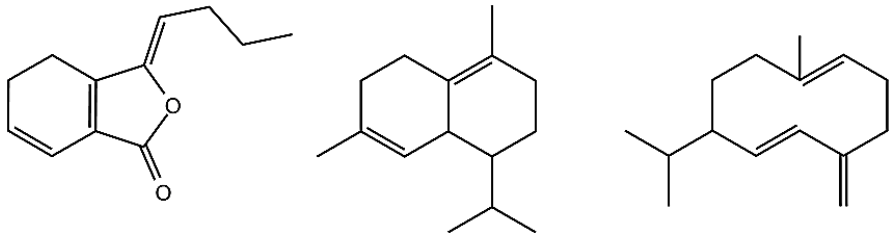
Table 1. Chemical composition of the essential oil of *K. odoratissima* fruits obtained by hydrodistillation method (Continued)

No.	Compound name[a]	%	RI[b]
21	Alloaromadendrene	1.81	1487
22	*γ*-Amorphene	1.07	1492
23	*α*-Selinene	1.84	1498
24	*α*-Muurolene	1.78	1504
25	*γ*-Cadinene	2.80	1512
26	*δ*-Cadinene	12.26	1521
27	Selina-3,7(11)-diene	2.16	1532
28	Neryl 2-methyl butanoate	4.81	1571
29	Germacrene B	4.02	1556
30	Spatulenol	0.56	1582
31	Salvial-4(14)-en-1-one	0.27	1599
32	*α*-epi-Muurolol	0.47	1648
33	3-Butylphthalide	0.61	1658
34	(*Z*)-Butylidene phthalide	3.05	1678
35	(*Z*)-Ligustilide	15.93	1797
36	(*E*)-Ligustilide	0.35	1741
	Monoterpene hydrocarbons	3.67	
	Oxygenated monoterpenes	9.77	
	Sesquiterpene hydrocarbons	53.68	
	Oxygenated sesquiterpenes	1.30	
	Non-terpenes	22.32	
	Total identified	90.74	

[a] Compounds listed in order of elution from BPX5 column; [b] Retention indices to C_8-C_{24} *n*-alkanes on BPX5 column.



(*Z*)-Ligustilide δ-Cadinene Germacrene D

Fig. 2. The structures of main compounds identified in essential oil of *K. odoratissima* fruits

4. Discussion

Five flavonol 3-O-glycosides, namely, isorhamnetin 3-O-*β*-D-glucopyranoside (**1**), quercetin 3-O-*β*-D-glucopyranoside (isoquercetin) (**2**), isorhamnetin 3-O-*β*-D-rutinoside (narcissin) (**3**), isorhamnetin 3-O-*β*-D-glucuronide (**4**) and quercetin 3-O-*β*-D-glucuronide (mequilianin) (**5**) were isolated from the fruits of *K. odoratissima* in present study for the first time.

Previously, two steroid derivatives, stigmasterol and *β*-sitosterol and one phthalide, 3-butyliden-4,5-dihydrophthalide reported from *n*-hexane extract of *K. odoratissima* fruits [14]. Flavonoids are phenolic compounds with well-known free radical scavenging activity which makes them appropriate food supplements to prevent oxidative stress related diseases such as cancers, diabetes, cardiovascular, inflammatory

and neurodegenerative diseases (such as Alzheimer and Parkinson) [22].

The potent antioxidant activity of isorhamnetin 3-O-*β*-D-rutinoside, a major flavonol 3-O-glycoside isolated in present study, has been reported in DPPH (IC_{50}; 9.01 μM, IC_{50} value of ascorbic acid as positive control; 11.93 μM) and $ONOO^-$ (IC_{50}; 2.56 μM, IC_{50} value of DL-penicillamin as positive control; 5.1 μM) methods [23]. This compound (isorhamnetin 3-O-*β*-D-rutinoside) has also been reported as one of antimicrobial principles of *Atriplex halimus* aerial parts with considerable antibacterial activity against *Streptococcus pyogenes* (inhibition zone; 17 ± 0.09 mm), *Escherichia coli* (inhibition zone; 16 ± 0.09 mm) and *Acinetobacter baumanii* (inhibition zone; 16 ± 0.13 mm), having a low cytotoxicity on PMNCs (IC_{50}; 450 μg ml^{-1}) [24].

Isorhamnetin 3-O-*β*-D-glucopyranoside and quercetin 3-O-*β*-D-glucopyranoside (isoquercetin), two other flavonol glycosides isolated from *K. odoratissima* fruits in present study, have been documented for its antioxidant [25], antidiabetic [26] and hepatoprotective [27] activities. Furthermore, in a bioassay-guided fractionation study quercetin 3-O-glucoside was isolated as active compounds of *Sambucus ebulus* L. leaves with rremarkable wound healing activity [28].

Isorhamnetin and quercetin were also isolated as their 3-O-*β*-D-glucuronide derivatives in the present study. A bioactivity-guided isolation study reported the isolation of isorhamnetin 3-O-*β*-D-glucuronide as one of active compounds of *Sanguisorba officinalis* L. (Rosaceae) with considerable lipid accumulation inhibition on 3T3-L1 cells (IC_{30}; 18.43 μM) [29]. *Chuquiraga spinosa* (Asteraceae), *Cichorium spinosum* L. (Asteraceae), *Foeniculum vulgare* L. (Apiaceae) and some cultivars of *Vitis vinifera* L. (Vitaceae) are examples of plants reported to contain isorhamnetin 3-O-*β*-D-glucuronide [30-34].

Quercetin 3-O-*β*-D-glucuronide has been found in some plant species such as *Calligonum comosum* L. (Polygonaceae), *Rubus ulmifolius* Schott. (Rosaceae) and *Phaseolus vulgaris* L. (Fabaceae), as well as one of major human metabolites of quercetin [35-38]. One study suggested that supplementation of quercetin 3-O-*β*-D-glucuronide can reduce the relative risk for developing Alzheimer's disease (AD) dementia [39]. They showed that quercetin 3-O-*β*-D-glucuronide can significantly reduce the generation of *β*-amyloid (A*β*) peptides and improves AD-type deficits in hippocampal formation basal synaptic transmission and long-term potentiation, possibly through mechanisms involving the activation of the c-Jun N-terminal kinases and the mitogen-activated protein kinase signaling pathways [39]. In another study, quercetin 3-O-*β*-D-glucuronide showed to possess anti-neuroinflammatory activity in lipopolysaccharide (LPS)-induced inflammation in BV2 cells by inhibition of the production of NO and PGE2 and reducing the levels of some pro-inflammatory cytokines such as TNF-*α* and interlukin-1*β* [40]. Moreover, beneficial effects of quercetin 3-O-*β*-D-glucuronide in arteriosclerosis prevention has been reported via elevating plasma HDLC followed by induction of ABCA1 (ATP-binding cassette, subfamily A, member 1) expression, a crucial cholesterol transporter involved in reverse cholesterol transport [41].

In the present study, GC-MS analysis of the essential oil extracted from the fruits of *K. odoratissima* resulted in the identification of thirty six compounds, of which (*Z*)-ligustilide (15.93 %), *δ*-cadinene (12.26 %) and germacrene D (12.18 %) were the main compounds (Fig. 2). One study reported *α*-caryophyllene (22.60 %), *α*-humulene (20.0 %) and cyclopropane (11.54 %) as the main compounds of the essential oil of *K. odoratissima* fruits, gathered from Zardkooh

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Mountain, (Charmahal-Bakhtiari, Iran) [42]. In another study on the fruits of this plant collected from Samsami region (Chaharmahal-Bakhtiari, Iran) (*Z*)-Ligustilide (86.0 %), (2*E*)-decen-1-ol (8.0 %), pentyl cyclohexa-1,3-diene (4.4 %) were characterized as main compounds of its essential oil [43]. The results of previous reports on essential oil composition of different parts of *K. odoratissima* have been summarized in Table 2 [5, 7, 8, 13, 15, 43-47]. Beside possible genetic diversity, variation observed in essential oil constituents may be raised from some geographical differences between the populations of *K. odoratissima* [48].

Table 2. The results of essential oil analysis of *K. odoratissima* from the previous and present studies.

Location of collection	Date	Method	Part	Main compounds (%)	Ref.
Fereydunshahr, Isfahan, Iran	Dec 2018	HD[a]	Fruit	(*Z*)-Ligustilide (15.9 %), *δ*-Cadinene (12.3 %), Germacrene D (12.2 %)	Present study
Dishmook, Kohgiluye-Buyer Ahmad, Iran	May 2014	HD	Leaf	(*Z*)-Ligustilide (58.7%), Carvacrol (7.8%), *trans*-3-Butylidene phthalide (4.9%), Thymol (4.5%),	5
Keloseh region, Isfahan, Iran				(*Z*)-Ligustilide (53.5%), Thymol (7.9%), *trans*-3-Butylidene phthalide (3.3%)	
Fereydunshahr, Isfahan, Iran				(*Z*)-Ligustilide (51.3%), Thymol (8.7%), Carvacrol (3.2%)	
Central Zagros Mountain, Chaharmahal-Bakhtiari, Iran	Apr 2009	HD	Leaf	(*Z*)-Ligustilide (77.7%), 2-Octen-1-ol acetate (6.3%), (*E*)-Ligustilide (2.3%)	7
Kohgiluye-Buyer Ahmad, Iran	Apr-May 2012	HD	Aerial part	(*Z*)-Ligustilide (34.5%), (*E*)-Ligustilide (11.8%), 3-(*Z*)-Butylidenephthalide (8.8%), Dec-9-en-1-ol (5.9%)	8
Central Zagros mountain, Iran	Mar 2006	HD	Aerial part	(*Z*)-Ligustilide (85.9%), *α*-Copaene (1.4%), *δ*-Cadinene (0.7%)	13
Fereydunshahr, Isfahan, Iran	Aug 2009	HD	Fruit	*Z*-ligustilide (51.0%), *δ*-Terpinen-7-al (10.3%), *δ*-terpinene (5.3%), Cumin aldehyde (5.2%)	15
Zardkooh Mountain, Charmahal-Bakhtiari, Iran	Jul-Aug 2011	HD	Fruits	*α*-Caryophyllene (22.6%), *α*-Humulene (20.0%), Cyclopropane (11.5%)	42
Samsami region, Chaharmahal-Bakhtiari, Iran	Jun-Jul 2014	HD	Root	(*Z*)-Ligustilide (54.0%), (2*E*)-Decen-1-ol (10.7%), Pentyl cyclohexa-1,3-diene (6.4%), (3*Z*)-Butylidene phthalide (5.8%)	43
			Stem	(*Z*)-Ligustilide (58.7%), (2*E*)-Decen-1-ol (11.6%), Pentyl cyclohexa-1,3-diene (4.4%)	
			Leaf	(*Z*)-Ligustilide (66.8%), (2*E*)-Decen-1-ol (12.3%), Pentyl cyclohexa-1,3-diene (3.8%)	
			Flower	(*Z*)-Ligustilide (62.4%), Geranyl butyrate (9.0%), *trans*-Muurola-4(14) 5-diene (5.5%)	
			Fruit	(*Z*)-Ligustilide (86.0%), (2*E*)-Decen-1-ol (8.0%), Pentyl cyclohexa-1,3-diene (4.4%)	

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Table 2. The results of essential oil analysis of *K. odoratissima* from the previous and present studies (Continued)

Location of collection	Date	Method	Part	Main compounds (%)	Ref.
Keloseh region, Fereydunshahr, Isfahan, Iran	Aug 2007	HD	Stem	Borneol (36.9 %), Bornyl acetate (14.0 %), 1,8-Cineol (13.6 %), Camphor (9.5 %)	44
			Flower	1,8-Cineol (22.0%), Camphor (20.1%), *α*-Pinene (19.0%), Camphene (12.0%), Bornyl acetate (5.8 %)	
			Leaf	*β*-Terpinene (23.0%), Sabinene (9.0%), *α*-Thujene (8.4%)	
Fereydunshahr, Isfahan, Iran	Jul 2007	HD	Aerial part	(*Z*)-Ligustilide (87.6%), (*E*)-Ligustilide (3.2 %), Piperitone epoxide (3.1%)	45
Bazoft region, Chaharmahal-Bakhtiari, Iran	April 2008	HD	Aerial part	(*Z*)-Ligustilide (47.3%), (3*E*)-Butyldiene phthalide (17.38%), (*E*)-Ligustilide (6.3%), 2-Octen-1-ol acetate (5.4%)	46
Koohrang region, Chaharmahal-Bakhtiari, Iran				(*Z*)-Ligustilide (33.7%), (3*E*)-Butyldiene phthalide (20.1%), (*E*)-Ligustilide (6.6%), 2-Octen-1-ol acetate (5.2%)	
Samsami region, Chaharmahal-Bakhtiari, Iran				(*Z*)-Ligustilide (37.6%), (3*E*)-Butyldiene phthalide (19.9%), (*E*)-Ligustilide (7.0%), Kessane (5.3%)	
Zagros mountain, Iran	May-Jun 2012	HD	Aerial part	*α*-Pinene (20.1%), 1,8-Cineole (18.2%), (*Z*)-Ligustilide (15.5%)	47

(*Z*)-Ligustilide, a phthalide derivative identified as major constituent in most of previously studied *K. odoratissima* essential oils, has received attention for its interesting pharmacological and biological effects such as neuroprotective, anti-oxidation, anti-inflammatory, analgesic and anticancer effects [49].

5. Conclusion

The presence of flavonoid glycosides (**1-5**) and phthalide derivatives with known health beneficial effects make the fruits of *K. odoratissima* as a natural remedy with valuable therapeutic potentials and suggest it an appropriate option for further studies. Meanwhile, restricted distribution of *K. odoratissima* underline the importance of an appropriate conservation approach for the uses of this species for medicinal and food purposes.

Author contributions

MK: Study supervision and data interpretation; SG: Experimental analysis and preparation of manuscript draft; GS: Experimental analysis; MD: Original idea presentation, study design, study supervision, data interpretation and revision of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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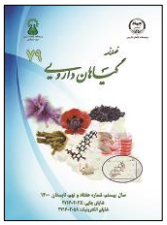

مقاله تحقیقاتی

ترکیبات فیتوشیمیایی میوه کلوس (*Kelussia odoratissima* Mozaff.)، گیاهی معطر و انحصاری ایران

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اطلاعات مقاله

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چکیده

مقدمه: گیاه کلوس (*Kelussia odoratissima* Mozaff.) از تیره چتریان، گیاهی است علفی و پایا که به صورت انحصاری در غرب ایران می‌روید. مردم بومی از بخش‌های هوایی معطر این گیاه به صورت سنتی به عنوان عطر و طعم‌دهنده در تهیه بعضی غذاهای محلی و همچنین در درمان بیماری‌های مختلف استفاده می‌کنند. **هدف:** مطالعه حاضر با هدف جداسازی و شناسایی ترکیبات فنولی و همچنین آنالیز ترکیبات اسانس میوه‌های گیاه کلوس طراحی شده است. **روش بررسی:** ترکیبات فراکسیون بوتانولی نرمال بدست آمده از عصاره هیدروالکلی میوه گیاه با استفاده از کروماتوگرافی روی ستون‌های فاز نرمال و سفادکس ال اچ-۲۰ مورد جداسازی و خالص‌سازی قرار گرفت. ترکیبات اسانس بدست آمده از نمونه نیز با استفاده از تکنیک کروماتوگرافی گازی متصل به طیف‌سنج جرمی آنالیز شد. **نتایج:** مطالعه فیتوشیمیایی روی میوه‌های گیاه کلوس به جداسازی و شناسایی پنج گلیکوزید فلاونولی با نام‌های ایزورامنتین ۳-اُ- گلوکوزید (**۱**)، کوئرستین ۳-اُ- گلوکوزید (ایزوکوئرستین) (**۲**)، ایزورامنتین ۳-اُ- روتینوزید (نارسیسین) (**۳**)، ایزورامنتین ۳-اُ- گلوکورونید (**۴**) و کوئرستین ۳-اُ- گلوکورونید (مِکوئیلینانین) (**۵**) انجامید. تعداد سی و شش ترکیب نیز در نتیجه آنالیز اسانس حاصل از میوه‌های گیاه شناسایی شد که از این میان ترکیبات (Z)- لیگوستیلید (۱۵/۹۳ درصد)، دلتا- کادینن (۱۲/۲۶ درصد) و جرماکرن دی (۱۲/۱۸ درصد) ترکیبات عمده بودند. **نتیجه‌گیری:** نتایج این مطالعه، میوه‌های گیاه کلوس را به عنوان منبعی از گلیکوزیدهای فلاونوئیدی و مشتقات فتالات معرفی می‌کند. حضور این ترکیبات که خواص بیولوژیک و اثرات سودمند آنها بر سلامتی در مطالعات پیشین نشان داده شده است، میوه‌های این گیاه را به عنوان گزینه مناسبی برای مطالعات مرتبط بیشتر مطرح می‌کند.

مخففها: NMR، رزونانس مغناطیسی هسته‌ای؛ GC-MS، کروماتوگرافی گازی متصل به طیف‌سنج جرمی؛ TLC، کروماتوگرافی لایه نازک

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