

## The essential oil composition of wild celery (*Kelussia odoratissima* Mozaff.): An Iranian endemic plant

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### ABSTRACT

**Background and aims:** *Kelussia odoratissima* Mozaff. is an Iranian endangered endemic plant with a wide use in the Central Zagros region of Iran as spice and medicinal herb for inflammatory and cardiovascular purposes. The aim of this study was to assess the essential oil compositions of Birahgan ecotype of *K. odoratissima* in Chaharmahal and Bakhtiari province.

**Methods:** The aerial parts of *K. odoratissima* were collected from Birahgan ecotype and then dried. The essential oils were obtained by hydro-distillation and were analyzed by Gas Chromatography-Mass Spectrometry.

**Results:** Twenty-four compounds were identified, of which the major components were found to be Z-ligustilide (64.3%), 2-octen-1-ol acetate (12.0%), (Z)-3-butyldiene Phthalides (4.5%), menthyl acetate (3.0%),  $\alpha$ -copaene (2.8%),  $\delta$ -cadinene (2.2%), neo-menthol (1.6%), menthol (1.5%) and  $\alpha$ -cadinene (1.5%). The group of Phthalides was included as Z-ligustilide, E-ligustilide, (Z)-3-Butyldiene Phthalides and (E)-3-Butyldiene Phthalides, which these compounds formed 70.10% of all identified components.

**Conclusion:** Like other ecotypes, the ecotype of Birahgan is a rich source of Z-ligustilide that can be used in the pharmaceutical industries.

**Keywords:** Birahgan, Central Zagros, Essential oil, Z-ligustilide, *Kelussia odoratissima* Mozaff.

Original article

### INTRODUCTION

Iran is known as one of the main regions of plant biodiversity in the world with a desirable climate and geography. This allows a rich diversity of medicinal plants that are mainly endemic.<sup>1</sup> *Kelussia odoratissima* Mozaff, known as Keluss or Karafs-e- Bakhtiari in Persian, with only one species founded merely in Central Zagros Mountain belonged to Apiaceae family is a sweet-smelling, self-growing plant, umbelliferae, wild rebus, erect, hairless, annual aromatic herb, which grows up to

120 to 200 cm high.<sup>2-6</sup> It has enormous uses as food, spice and medicinal herb.<sup>7</sup> *Kelussia odoratissima* Mozaff from Apiaceae family is an endemic and endangered ethnomedicinal plant from Iran.<sup>8</sup> It bears a unique flavorful smell; *K. odoratissima* is vastly used as spice especially in yoghurt. Pickles of small leaves and shoots are common in Chaharmahal and Bakhtiari province in south west of Iran.<sup>9</sup> The leaves of this plant can be used as an edible, wild vegetable, flavoring and it is traditionally consumed as

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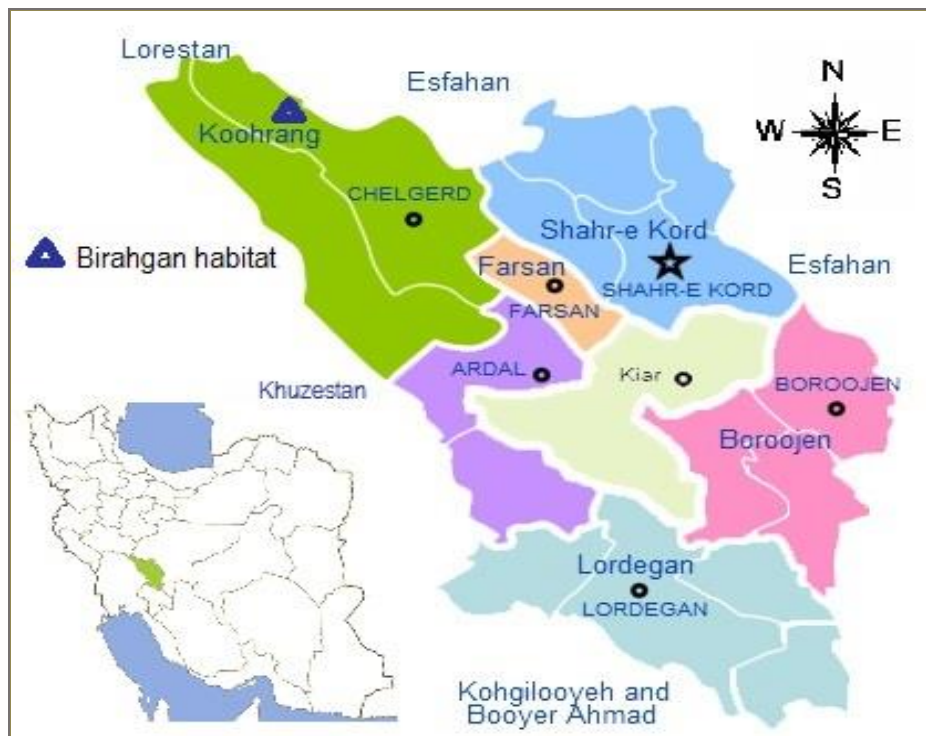
a medicinal plant to treat hypertension, inflammation, ulcer and cardiovascular diseases.<sup>2</sup> Species traditionally have anti-ache, anti -inflammation and anti - cough effects. Seed and root of these species were boiled and used to cure the common cold and intensive coughs and its organs are been used to treat the stomachache, rheumatism and blood refining.<sup>6</sup> It was also used as food additives in traditional preparations such as pickles.<sup>4</sup> The flavonoid compounds as a main part of the plant has anti-inflammatory, anti-viral, anti-diabetic, anti-cancer and anti-toxin, anti-stress effects, antioxidant, and antihyperlipidemic properties, and ulcerative colitis, sedative property, antibacterial effect, larvicidal activity, antibacterial agent, pulmonary hypertension, anti-tumor and insecticidal properties.<sup>5,7,8,10-18</sup> Results of a study by Asadiyeh Shojaei et al. showed that

the major composition of essential oil of aerial parts of 3 ecotypes (Koohrang, Bazoft and Samsami) of *K. odoratissima* is phthalides (such as Z-ligustilid) that have a positive impact on the nervous system, blood pressure, and cholesterol.<sup>19</sup>

There is no report on assessment of chemical compounds of *K. odoratissima* in the ecotype of Birahgan. The aim of this study was to investigate the essential oil compositions of Birahgan ecotype of *K. odoratissima* in Chaharmahal and Bakhtiari province.

## METHODS

The studied region is located in Koohrang county, Chaharmahal and Bakhtiari province, in Central Zagros of Iran (Figure 1).



**Figure 1:** The location of Birahgan habitat in Iran

The studied region is located at 49° 54' 42 East longitude and 32° 39' 12 North

latitude (elevation 2320 m above sea level). Annual rainfall is about 1441 mm/year based

on a neighbor station that often consists of snow. The aerial parts of *K. odoratissima* were collected in May 2013. Harvested parts were dried at room temperature and shaded for one week. Then, the dried parts of the plant were subjected to household coffee grinder in order to turn them into a coarse powder to use in our experiment.

The ground plant material (100 g) was subjected to hydrodistillation (1000 ml distilled water) for 3 h using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (1988). Samples were dried with anhydrous sodium sulfate and kept at 4°C until analysis.

The GC/MS analysis was carried out using an Agilent 5975 C gas chromatograph (column HP-5MS, 30 m long × 0.25 mm i.d. and film thickness 0.25 µm). Initial temperature of the oven was 50°C, holding time for 3 min, followed by temperature enhancement at the rate of 8°C/min to reach final temperature of 200°C. Then, heated up to 290°C at the rate 12°C/min and holding time for 3 min. Injector temperature was 280°C, and helium was used as a carrier gas with a flow rate of 1.2 ml/min. The spectra were recorded on an Agilent 5975 mass spectrometer at 70eV (ionization voltage) and the electron ionization mode was applied. The source ionization temperature was 150°C and the transfer line temperature was 280°C.

Retention indices of all the components were determined by Kovats method using n-alkanes as standards. Identification of individual components was made by comparison of their retention times with those of available analytical standards and by computer search, matching mass spectral data with those held in Nist and Wiley library of mass spectra and compared with constituents known from the literature.<sup>20</sup>

## RESULTS

Twenty four compounds were identified from the Birahgan ecotype of *K. odoratissima* using the hydrodistillation method. The list of volatile compounds is presented in Table 1.

**Table 1:** Compositions of essential oil of Birahgan in *Kelussia odoratissima* Mozaff.

No.	Compound	KI	%
1	Limonene	1030	0.3
2	Terpinolene	1090	0.2
3	Neo-menthol	1160	1.6
4	Menthol	1167	1.5
5	Unknown	1279	12.0
6	Menthyl acetate	1295	3.0
7	α-cubebene	1354	0.3
8	α-copaene	1382	2.8
9	β-caryophyllene	1427	1.0
10	E-β-farnesene	1452	0.1
11	α-humulene	1462	0.4
12	β-acoradiene	1474	0.1
13	Allo-aromadendrene	1482	0.6
14	Germacrene-D	1489	0.9
15	β-bisabolene	1502	tr
16	β-himachalene	1509	tr
17	Cuparene	1515	0.4
18	(Z)-γ-bisabolene	1521	1.0
19	δ-cadinene	1529	2.2
20	α-cadinene	1537	1.5
21	(Z)-3-Butyldiene Phthalide	1692	4.5
22	(E)-3-Butyldiene Phthalide	1739	1.1
23	Z-ligustilide	1757	64.3
24	E-ligustilide	1840	0.2
	Total		99.9

tr= trace amount (<0.1%); KI= Kovats Index.

The major volatile compounds were Z-ligustilide (64.3%), 2-octen-1-ol acetate (12.0%), (Z)-3-butyldiene phthalide (4.5%), menthyl acetate (3.0%), α-copaene (2.8%), δ-cadinene (2.2%), neo-menthol (1.6%), menthol (1.5%) and α-cadinene (1.5%) containing 93.4% of total identified components.

Generally, the results revealed that the highest percentage of the compound in the habitat of Birahgan was Z-Ligustilide. Our

result is in accordance to those obtained by recent investigations.<sup>21</sup>

The chemical class distributions of the volatile compounds are given in Table 2. The compounds were separated into four classes including esters, monoterpene

hydrocarbons, sesquiterpene hydrocarbons and phthalides. The highest and the lowest associated values were phthalides (70.10) and sesquiterpene hydrocarbons (11.30), respectively.

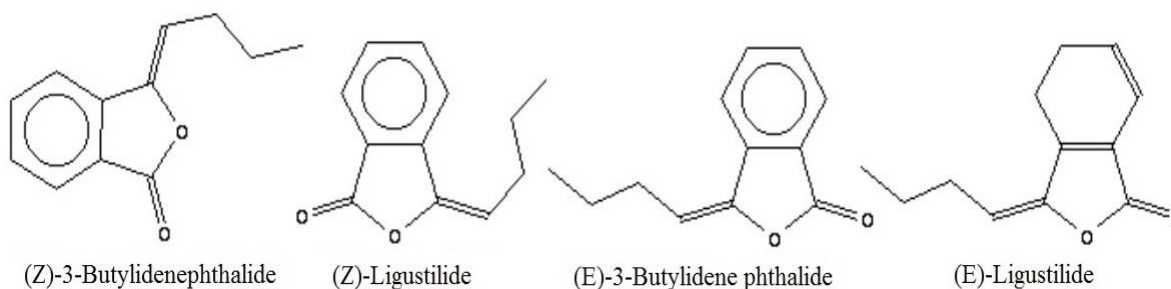
**Table 2:** The classification of chemical compounds of *K. odoratissima* in Birahgan ecotype

Chemical class	%	Number of compounds
Esters	12.00	1
Monoterpene Hydrocarbons	18.60	5
Sesquiterpene Hydrocarbons	11.30	14
Phthalides	70.10	4

## DISCUSSION

The aim of this study was to investigate the essential oil compositions of Birahgan ecotype of *K. odoratissima* in Chaharmahal and Bakhtiari province.

According to the present results, Phthalides were including Z-ligustilide, E-ligustilide, (Z)-3-Butyldiene Phthalides and (E)-3-Butyldiene Phthalides (Figure 2).



**Figure 2:** Chemical structure of Phthalides

Phthalides and their corresponding derivatives are found as constituents of several genera within the family of Apiaceae which have been reported to possess a variety of ethnobotanical applications and therapeutic effects as sedative, antioxidant, larvicidal activity, antigenotoxic or genoprotective, antiproliferative, fibrinolytic, analgesic and anti-inflammatory effects.<sup>2,5,11,15,22-24</sup> Effect on learning and memory, gastric acid and pepsin secretion level, Anti-hyperlipidemia effect has been reported.<sup>13,25-27</sup> Z-ligustilide was the major compound that has been reported to have a positive impact on the

nervous system, blood pressure, and cholesterol.<sup>21</sup>

In our study, the major volatile compounds were Z-ligustilide (64.3%), 2-octen-1-ol acetate (12.0%), (Z)-3-butylidene phthalide (4.5%), menthyl acetate (3.0%),  $\alpha$ -copaene (2.8%),  $\delta$ -cadinene (2.2%), neo-menthol (1.6%), menthol (1.5%) and  $\alpha$ -cadinene (1.5%) containing 93.4% of total identified components, while in a study by Ghasemi Pirbalouti, it was shown that the main constituents of the essential oils were 3-n butyl phtalide (34.1 to 45.6%), neophyadiene (42.2 to

68.2%), e3-tetradecenylacetate (1.1-5.2%), butylidene phthalide (1.1 to 9.2%), hexadecanoic (1.1 to 5.3%), and 6-butyl-1,4-cycloheptadin (1.1 to 6.1%)<sup>2</sup> that is said that this difference in the content of compounds is due to the region of sample collection.

Z-ligustilide simplifies blood circulation of rats and mice, penetrates the blood brain barrier to restrict ischemic brain damage and relieve pain. Preclinical studies have indicated that z-ligustilide may also relax smooth muscle in the circulatory, respiratory and gastrointestinal systems.<sup>28</sup>

In another study, it was suggested that the different organs of *K. odoratissima* are potential sources of Phthalides are known for their anti-inflammatory, anti-tumor and insecticidal properties.<sup>18</sup>

The flavonoid compounds as a main part of the plant has anti-inflammatory, anti-viral, anti-diabetic, anti-cancer and anti-toxin, anti-stress effects, Antioxidant, and antihyperlipidemic properties and ulcerative colitis, sedative property, antibacterial effect, larvicidal activity, antibacterial agent, anti-tumor and insecticidal properties.<sup>5,7,8,10-16,18</sup>

The aerial parts of *K. odoratissima* was shown to have 27 components. The main constituents of the essential oil are phthalides including 3-butylidene-4,5-dihydrophthalide (z-Ligustilide) (85.9%), cis-3butylidene phthalide (0.4%), 3 N butyl phthalide (0.3%),  $\alpha$ -copaene (1.4%) and  $\delta$ -cadinene (0.7%).<sup>11</sup> In a study by Sajjadi et al., thirty eight compounds of the essential oil composition of the fruits of this plant were identified, that the most important of all were Z-ligustilide (29.2%), germacrene-B (15.9%) and germacrene-D (15.5%).<sup>9</sup>

In a study by Esmaili et al., the essential oils of the aerial parts (flower, stem and leaf) of *Kelussia odoratissima* Mozaff. Were examined and it was concluded that the main components of the stem were identified as being borneol (36.9 %), bornyl

acetate (14.0 %) and <sup>1,8</sup> cineol (13.6 %) and in the flower were identified as 1,8-cineol (22.0 %), camphor (20.1 %),  $\alpha$ -pinene (19.0 %) and camphene (12.0 %). In the leaf oil of the plant  $\beta$ -terpinene (23.0 %) was the predominant compounds.<sup>29</sup>

Essential oil compositions of aerial parts of the three ecotypes (Koohrang, Bazoft, and Samsami) of wild celery (*Kelussia odoratissima*) were determined by Shojae et al. 22 constituents were identified for Bazoft ecotype, of which the major ones were such as z-ligustilide (47.31%) and 3-e-butyl phthalide (17.38%). 24 compounds were identified for the Koohrang ecotype, the main compounds composed of z-ligustilide (33.73%), and 3-e-butyldiene phthalide (20.1%). 24 compounds for the Samsami ecotype were reported. Its main compounds were z-ligustilide (37.55%) and 3-e-butyl phthalide (19.92%). z-ligustilide was the major compound that has been reported to have a positive impact on the nervous system, blood pressure, and cholesterol.<sup>21</sup>

The main constituents of the volatile oil are Phthalides with z-ligustilide as the major constituent.<sup>11</sup> Non-volatile components of *K. odoratissima* were identified in another study.<sup>5,22</sup>

The larvicidal activity of essential oil of *K. odoratissima* was evaluated in a study and forty-nine constituents were identified in the oil. The main constituents of the oil were Z-ligustilide (77.73%), 2-octen-1-ol acetate (6.27%), E-ligustilide (2.27%) and butylidene phthalide (1.97%) and it was illustrated that *K. odoratissima* oil has potential source of larvicidal compounds.<sup>15</sup>

The antigenotoxic or genoprotective effect of aqueous and methanolic extracts of *K. odoratissima* against DNA damage caused by MMS was observed. Therefore, the use of *K. odoratissima* can probably prevent the complications of these damages.<sup>23</sup>

## CONCLUSION

*K. odoratissima* Mozaff., is an endemic and rare medicinal plant of Iran which is extremely endangered due to increased harvesting and unsuitable collection practices throughout wild habitats. Up to 70% of essential oil compositions of *K. odoratissima* belong to the phthalides. Due to the high amount of Z-ligustilide, it can be concluded that the herb and essential oil of *K. odoratissima* can be used in the pharmaceutical industries. Besides, further studies are necessary to explore the biological activities of its components.

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