Chemical composition of the essential oil of wild and cultivated plant populations of *Kelussia odoratissima* Mozaff.

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*Kelussia odoratissima* Mozaff. is an endemic plant in Iran, which has been used as a sedative and vegetable in Bakhtiari folk. The plant is known as Keluss or Karafs-e-Bakhtiari in Persian. The wild and cultivated plant populations of *K. odoratissima* collected throughout two provinces (Isfahan and Chaharmahal va Bakhtiari), Southwest Iran, were examined for chemical variability in leaves for components. The essential oils of *K. odoratissima* of eighteen populations were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The results revealed that differences in the content of compounds depending on region of sample collection. The main constituents of the essential oils were 3-n butyl phthalide (34.1 to 45.6%), neophyadiene (42.2 to 68.2%), e3-tetradeceneylacetate (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%). It can be concluded that two main chemotypes (3-n butyl phthalide and neophyadiene) of essential oils of *K. odoratissima* may be identified by the characteristic concentrations of the major compounds. The wild and cultivated populations of *K. odoratissima* were divided into five groups based on cluster analysis.

Key words: *Kelussia odoratissima* Mozaff., chemotype, essential oil, phthalides.

INTRODUCTION

*Kelussia* is one of the newest genera of Apiaceae (Umbellifera) family and is represented by only one species, *Kelussia odoratissima* Mozaff., which is found only in Iran (Mozaffarian, 2003). It is a wild rebus, erect, glabrous, perennial aromatic herb, which grows to height of 120 to 200 cm. The flowers are 1 to 2 mm in diameter, all hermaphrodites. Petals are yellow and produced in compound umbels. In natural conditions, the plant is propagated through seeds that are in general produced once a year in late summer. This sweet-smelling, self-growing monotypic medicinal plant is endemic to a restricted area in western Iran. *K. odoratissima* is dispersed around some parts of the Zagros Mountains at 2500 m above sea level, including Isfahan, and Lorestan Provinces, and locally called “Kelus or Karafs-e-Bakhtiari” (Mozaffarian, 1996; Rabbani et al., 2011; Ghasemi Pirbalouti et al., 2007). The leaves of this plant used as an edible, wild vegetable, flavoring, indigestion and rheumatism with the ethnic communities of Bakhtyari and Chaharmahali (Figure 1), is found to be new in the literature of Iranian medicinal plants (Ghasemi Pirbalouti, 2009). Also, this plant is traditionally consumed as a medicinal plant to treat hypertension, inflammation, ulcers and cardiovascular diseases (Ahmadi et al., 2007). The areal parts of *K. odoratissima* have high nutritional values owing to their high minerals and vitamins (Asadiyeh Shojaei et al., 2011). The seeds have long been used as a remedy for various ailments, particularly to treat head cold and stomachache (Iravani and Jaberol-Ansar, 2005).

Currently, there are limited studies examining the pharmacological properties of this plant. For example, Chahrmahal va Bakhtiari, Kohkiloyeh va Boyerahmad recent publications on the antioxidant activity of
methanolic extract of *K. odoratissima* (Ahmadi et al., 2007), and the sedative property of essential oil and hydroalcoholic extract of *K. odoratissima* (Rabbani et al., 2011), which may be mediated by the bioactive phthalides contained in this plant (Kaufman et al., 1999). The results of recent study by Asadiyeh Shojaei et al. (2011) showed that major compositions of essential oil of aerial parts of the three ecotypes (Kooorhang, Bazoft and Samsami) of wild populations of *K. odoratissima* are z-Ligustilide and 3-e-butyl phthalide. Previous work (Omidbagi et al., 2008) showed that the essential oil from the aerial part of *K. odoratissima* contains 23 kinds of different valuable components, of which the major compound is z-Ligustilide. However, ligustilide and butyl phthalide are the major compounds in *K. odoratissima* essential oil, that has been reported to have a positive impact on the nervous system, blood pressure, and cholesterol (Kuang et al., 2008; Song et al., 2004).

The essential oil yield and their components in medicinal and aromatic plants is related to genetic, climate, edaphic, elevation and topography (Pourhi and Vyas, 2004; Rahimmalek et al., 2009) and genetyp (G), growing conditions (E) and their interaction (G x E) (Basu et al., 2009). Chemical polymorphisms or chemotypes have been reported for many medicinal plants (Mockute et al., 2001; Russell and Southwell, 2003; Curado et al., 2006). Recent findings showed that some of the medicinal plant characteristics can be affected by genetic and ecological factors such as precipitation, temperature, plant competition and nitrogen content in the soil (Letchamo et al., 1995). However, there are no reports in assessment of chemical compounds of *K. odoratissima* essential oil in wild and cultivated populations. The aims of this study were (1) to investigate the chemical composition of *K. odoratissima* using GC/MS, (2) comparison of chemical compositions of essential oil of wild and cultivated plant populations of *K. odoratissima* collected throughout two provinces (Isfahan and Chaharmahal va Bakhtiari), Southwest Iran, (3) to assess the relationships between variations of chemical composition contents and the environmental factors involved in different geo-ecological regions.

**MATERIALS AND METHODS**

**Study site**

The study was carried out in Isfahan and Chaharmahal va Bakhtiari provinces, Iran (latitude from 31° to 32° N; longitude from 50° to 51° E). The natural vegetation is rangeland, oak forest and most of the areas are used for agriculture. The slope and elevation information were obtained from the Digital Elevation Model (DEM) using two well-known geographic information systems (GIS) software packages ILWIS (3.0 Academic) (Figure 2).

**Plant material**

The leaves and stems of wild and cultivated plant populations of *K. odoratissima* Mozaff were collected from Isfahan and Chaharmahal va Bakhtiari provinces, Central and Southwest of Iran. The samples of the plants were identified by regional floras and voucher specimens were deposited at the Herbarium of Agriculture and Natural Resources Researches Centre of Chaharmahal va Bakhtiari province, Iran (No: SHK 2611). The accessions of plants were transferred from natural habitats at the early growing stage on 15 April to 5 May 2010 (During 20 day's period).

**Sample preparation**

Harvested parts were dried at room temperature for one week, shade and ground into a powder. Air-dried and ground (50 mesh)
plant material was subjected to hydro-distillation (1000 ml distilled water) for 3 h using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988). Samples were dried with anhydrous sodium sulphate and kept in amber vials at 4°C until chromatographic analysis.

**GC analysis**

Oil obtained from *K. odoratissima* was analyzed using a Younglin Acme 6000 GC (Young Lin, Dongan-gu, Anyang-si, Kyounggi-do, Korea) with HP-5MS 5% phenylmethylsiloxane capillary column (30 m x 0.25 mm, 0.25 µm film thickness) equipped with an flame ionization detector (FID). Oven temperature was kept at 50°C for 5 min initially and then raised at the rate of 3°C /min to 240°C. Injector and detector temperatures were set at 290 and 300°C, respectively. Helium was used as carrier gas at a flow rate of 0.8 ml/min, and diluted samples (1/1000 in n-pentane, v/v) of 1.0 µl were injected manually in the splitless mode. Peaks area percents were used for obtaining quantitative data.

**GC/MS analysis**

**RESULTS**

The yellow oil of samples of eighteen populations of *K. odoratissima* was obtained by hydro-distillation in the yield of 0.30 to 0.35% (w/w). The chemical constituents were identified by GC and GC/MS, the results concerning the qualitative and quantitative analysis of the essential
oils are presented in the Table 1. The essential oils in the samples of wild populations of Koohrang, Lordegan and Feryadan were composed of 98, 98 and 83 identified compounds accounting for 88.3, 92.2 and 89.3% of the whole oil, respectively. The essential oils in the samples of cultivated populations of Koohrang, Lordegan and Feryadan were composed of 87, 113 and 69 identified compounds accounting for 88.7, 89.2 and 89.1% of the whole oil, respectively.

**DISCUSSION**

As previously reported (Rabbani et al., 2011; Ghasemi Pirbalouti et al., 2010), the yield (w/w) of the obtained essential oils ranged from 0.4 to 0.3%, based on dry weight. Asadiyeh Shojaei et al. (2011) reported that main compounds of essential oil of three ecotypes of *K. odoratissima* from Chahramahal va Bakhtiari were z-ligustilide (47.31%), 3-e- butyldiene phthalide (17.38%), e-ligustilide (6.33%); 2-octen-1-ol acetate (5.38%), caryoohylene oxide (4.11%), 3-n-butyldiene phthalide (3.14%), kessane (2.44%), pental benzene (1.84%), beta selinene (1.67%), and z-caryoohylene (1.43%) in Bazoft ecotype; z-ligustilide (33.73%), 3-e-butyldiene phthalide (20.1%), e-ligustilide (6.65%), 2-octen-1-ol, acetate (5.18%), beta-selinene (4.58%), kessane (4.09%), 3-n-butyldiene phthalide (3.57%), caraphyrene oxide (3.14%), cuparene (2.11%), pental benzene (1.74%), and e-beta- farnesene (1.27%) in Koohrang ecotype; z-ligustilide (37.55%), 3-e-butyldiene phthalide (19.92%), e-ligustilide (6.95%), kessane (5.34%), spathulenol (4.43%), 2-octen-1-ol, acetate (3.87%), globulol (3.56%), 3-n-butyldiene phthalide (3.24%), beta-selinene (2.59%), and pental benzene (1.22%) in Samsami ecotype. In other study (Rabbani et al., 2011), the main constituents of the essential oil were *K. odoratissima* from central Zagross mountains, Iran, were phthalides, which include 3-butyldiene-4, 5-

dihydrophthalide (z-Ligustilide) (85.9%), cis-3butyldiene phthalide (0.4%) and 3n butyl phthalide (0.3%).

Phthalides, and their corresponding dihydro and tetrahydro analogs, are components of several genera from the plant family Apiaceae (Beck and Chou, 2007). They are bioactive phytochemicals and have important molecular and cellular effects, which include the inhibition of DNMTs (DNA deoxyribonucleic acid methyltransferases) by targeting DNA hypermethylation (Yu and Wang, 2008), stimulation of glutathione transferases (Yand Wu, 2008), potential anti-fibrotic effects for the treatment and prevention of hepatic fibrosis (Lee et al., 2007) and protective effects for focal cortical ischemia in rats (Tian et al., 2005). Rabbani et al. (2011) reported the sedative property that *K. odoratissima* may be mediated by the bioactive phthalides contained in this plant. Compared with the previous report (Asadiyeh Shojaei et al., 2011; Rabbani et al., 2011), the content of neophyadiene and 3-n butyl phthalide in our samples were much higher. It can be concluded that two main chemotypes of essential oils of *K. odoratissima* may be identified by the characteristic concentrations of the major compounds. Polymorphism chemical polymorphisms or chemotypes have been reported for many medicinal plants. As chemical variation related to genetic and environmental factors is frequently encountered in aromatic plants (Pala-Paul et al., 2001). In order to determine and verify the variations of essential oils in different populations *K. odoratissima*, the composition data were analyzed by cluster analysis. Cluster analysis generated a dendrogram shown in Figure 3. The results show that wild populations of Lordegan are well separated from others. The dendrogram represents graphically the relationships among the populations and the groups, based on their essential oil composition. Five groups were formed by the Average Linkage cluster analysis. The first cluster encompassed *K. odoratissima*
accessions of three wild populations of Lordegan. The second cluster enclosed accession of two cultivated populations of Lordegan, two wild populations of Feraydan, and one wild population of Koohrang. The third cluster enclosed accession of one cultivated population of Lordegan and Koohrang, two wild populations of Koohrang, and one wild population of Feraydan. The fourth cluster enclosed accession of two cultivated populations of Koohrang. The fifth cluster enclosed accession of one cultivated population of Feraydan. In the present study, *K. odoratissima* with different chemical compositions have been reported. It is known that many factors influence the chemical constitution of *K. odoratissima* oil. The differences in the quantity or quality of the oils composition of the present and previous studies (Sezik and Basaran, 1985) may be because of the chemotypes, phonological stage, drying conditions, mode of distillation and geographic and climatic factors. Further studies are needed to see if the changes of chemical composition in the studied oils of *K. odoratissima* are on the account of different environmental conditions of both localities or the chemotypes are genetically fixed.

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