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Research Article

COMPARATIVE ANALYSIS OF CHEMICAL COMPOSITION OF *ACHILLEA WILHELMSII* FLOWERS IN PHENOLOGICAL DIFFERENT STAGES

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ABSTRACT
The *Achillea wilhelmsii* belongs to Asteraceae family. This plant is widely found in different regions of Iran and used for treatment of different diseases. The aim of this study was evaluated the chemical composition of *Achillea wilhelmsii* flowers in development different stages in Iran. The mature and immature flowers of plant collected from Chaharmahal-Bakhtiyari province (Saman) in Iran and they were analyzed by using GC/MS. The 23, 31compounds were identified in mature and immature flowers, respectively. The major components in flowers of mature were: 1,8-Cineole (35.532%), α-pinene (22.885%), Camphor (12.238%), Camphene (8.691%). The major components in flowers of immature were: α-pinene (36.227%), 1,8-Cineole (13.780%), Camphor (7.657%), Camphene (7.353%). In conclusion, this result showed that mature flowers have higher amounts of the 1,8-cineole compound, whereas, immature flowers have higher amounts of the α-pinene compound.

Keywords: *Achillea wilhelmsii, Essential Oils, GC/MS*

INTRODUCTION
Essential oils are volatile and liquid aroma compounds from natural sources, usually plants. Essential oils are not oils in a strict sense, but often share with oils a poor solubility in water. Essential oils often have an odor and are therefore used in food flavoring and perfumery. They are usually prepared by fragrance extraction techniques such as distillation, cold pressing, or extraction (maceration). Typically, essential oils are highly complex mixtures of often hundreds of individual aroma compounds.
Achillea, which belongs to the family Asteraceae and comprises more than 120 species. These plants are medicinal perennial herbs that are native to Europe and Western Asia, although they are also found in Australia, New Zealand and North America (Dokhani *et al.*, 2005). Several effects, such as anti-inflammatory, antihypertensive, and anti-hyperlipidemia and antitumor have been reported for *Achillea*. It is widely used in traditional medicine for gastrointestinal disorders and there are some reports of its effects, such as antispasmodic, choleretic, antiulcer, antibacterial (*Helicobacter pylori*), and hepatoprotective, on the gastrointestinal tract (Niazmand *et al.*, 2010). *Achillea wilhelmsii* C. Koch (Asteraceae) is widely found in different parts of Iran. This plant is full of flavonoids and sesquiterpene lactones, which have been shown to be effective in lowering blood lipids and hypertension (Asgari *et al.*, 2000), and widely used in Iranian traditional medicine for gastrointestinal disorders. It has chemical components, including flavonoids, alkaloids (achilleine), cineol, borneol, α- and β-pinene, camphor, car yophyllene, thujene, rutin, sesquiterpenoids, and monoterpenoids (Niazmand *et al.*, 2010).
Thus, the main aim of the present project was evaluated the chemical composition of *Achillea wilhelmsii* flowers in development different stages in Iran. In this study, *Achillea wilhelmsii* were collected in flower development different stages for evaluation of composition essential oil.

MATERIALS AND METHODS
Collection of Plants
The flowers of wild *Achillea wilhelmsii* C. Koch were collected in June, 2013 from Chaharmahal-Bakhtiyari (Saman) in Iran. The plants were identified by Dr. Feizi, Research Institue of Agriculture, Isfahan, Iran. The samples were separated and they were air-dried in shade at room temperature.

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Gas Chromatography-Mass Spectametry (GC-MS) Analysis

The chemical composition of the mature and immature flowers essential oil was analyzed using GC and GC-MS. The GC/MS analysis was carried out with an 20 Agilent 5975 GC-MSD system in research laboratory of Islamic Azad University, Khorasgan Branch, Isfahan, Iran. HP-5MS column (30m × 0.25mm. 0.25mm film thickness) 20 was used with helium as carrier gas (1.2mL/min). GC oven temperature was kept 20 at 50 C2 B0C for 3 min and programmed to 280 C2 B0C at a rate of 5 C2 B0C/min, and kept 20 constant at 290 C2 B0C for 3 min, at spilitless mode. The injector temperature was at 20 280 C2 B0C. Transfer 20 line temperature 280 C2 B0C. MS were taken at 70 20 eV. Mass ranger was from m/z 35 to 450. Head space GC-MS was used in this study. This method can use plant dry matter for chemical analysis.

RESULTS AND DICUSSION

GC-MS analysis of mature and immature flowers essential oil identified 23, 31 main compounds, respectively. The results obtained in our study showed that major compounds of *Achillea wilhelmsii* mature flowers were: 1,8-Cineole (35.532%), α-pinene (22.885%), Camphor (12.238%), Camphene (8.691%), (Table 1& Figure 1). The major compounds of *Achillea wilhelmsii* immature flowers were: α-pinene (36.227%), 1,8-Cineole (13.780%), Camphor (7.657%), , Camphene (7.353%), (Table 1& Figure 2). Therefore, the through terpene compounds in *Achillea wilhelmsii* flowers, the 1,8-Cineole content was highest in mature flowers and in *Achillea wilhelmsii* immature flowers α-pinene content was highest.

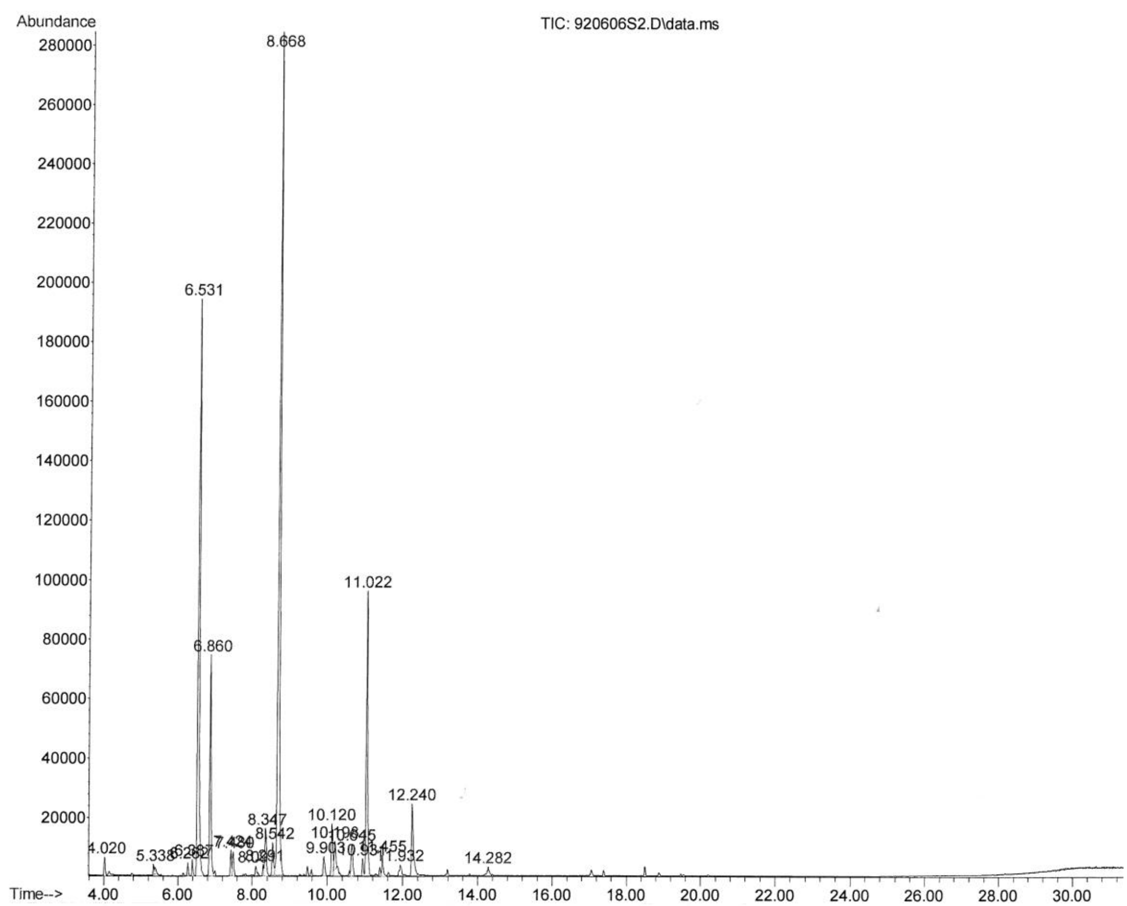

Figure 1: Typical GC-MS chromatogram of *Achillea wilhelmsii* mature flowers (Data is retention time for each component)

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Table 1: Chemical composition of *Achillea wilhelmsii* mature and immature flowers from Chaharmahal-Bakhtiyari

Compound	Mature flower (%)	Immature (%)	RT (Retention time)
α-Pinene	22.885	36.227	6.531
1,8-Cineole	35.532	13.780	8.668
Camphor	12.238	7.657	11.022
Camphene	8.691	7.353	6.860

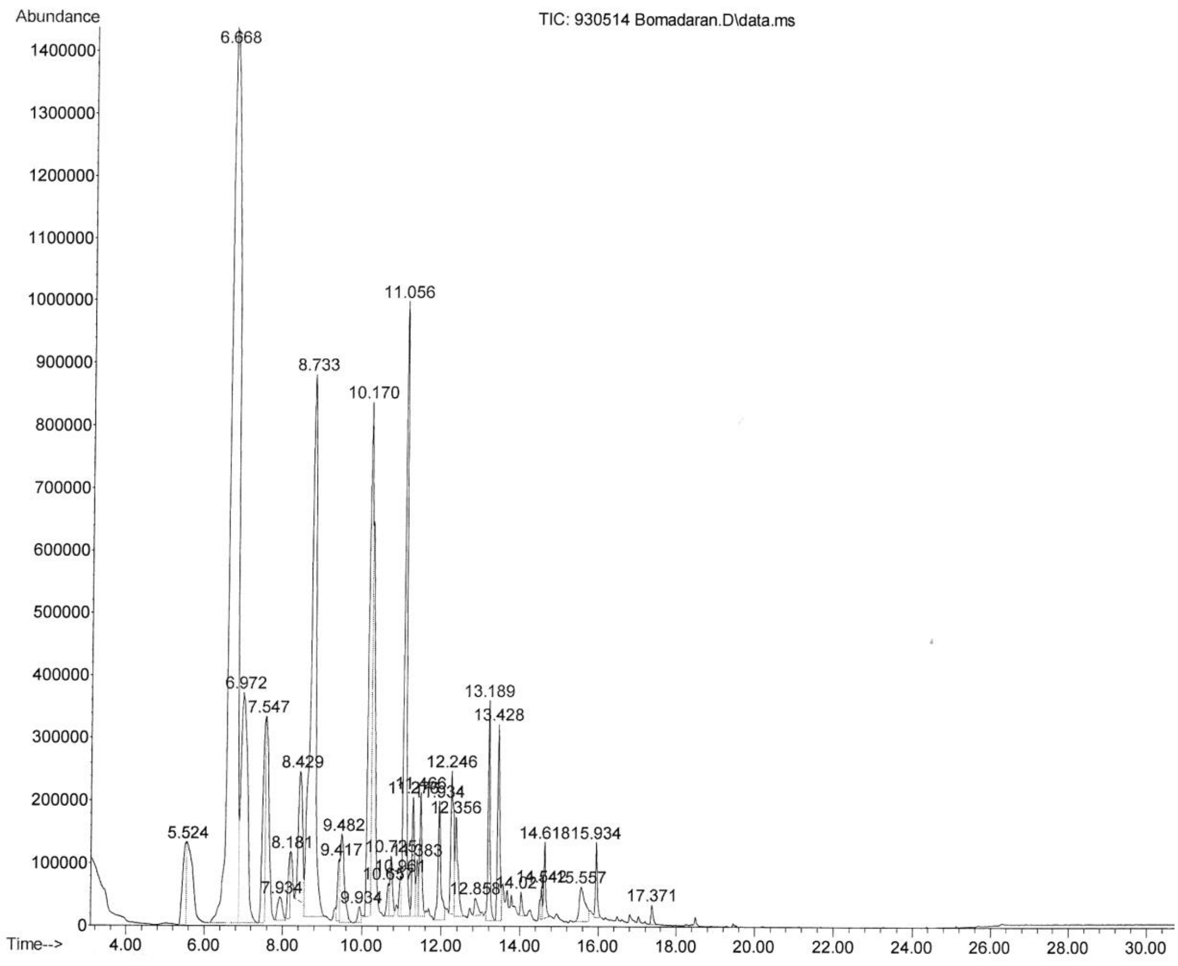

Figure 2: Typical GC-MS chromatogram of *Achillea wilhelmsii* immature flowers (Data is retention time for each component)

The results obtained in our study showed that twenty-three, thirty-one, compounds were identified in mature and immature flowers of *Achillea wilhelmsii* from Chaharmahal-Bakhtiyari, respectively. The our results in research of various phenological stages effects on essential composition of *Achillea wilhelmsii* observed that the highest density of 1,8-Cineole was related to mature flowers but the highest densities of α-pinene was obtained in immature flowers.

Dokhani *et al.,* (2005) analyzed *Achillea wilhelmsii* collected from Isfahan and Charmahal-Bakhtiyari province (Semirom, Gardaneh-Rokh, Lavark) in Iran and showed that the three ecotype of *Achillea wilhelmsii* contained **α-**pinene, camphene, β-pinene, **α**-thujene, limonene and 1,8-cineole. Dokhani *et al.*, study of headspace volatiles, camphor were detected in *A. wilhelmsii* from Lavark but not in that species from Gardeneh Rokh or Semirom. *A. wilhelmsii* from Semirom in Isfahan province showed the highest values for total phenol and tartaric ester. Golparvar and Hadipanah (2013) reported variation in essential oil content from *Thymus vulgaris* L. and *Salvia officinalis* L. collected from Isfahan climatic locations, so that, their results showed that the harvest time, ecological and climatical conditions can be influenced in essential oil composition. Nejad-Ebrahimi *et al.*, (2008) in study of Kermanian thyme (*Thymus caramanicus* Jalas.) observed that the lowest essential oil was obtained in vegetative phase. Jordan *et al.*, (2006) in research of various phenological stages effects on essential oil quality and quantity of *Himalayan thyme* (*Thymus hyemalis* Lange.) observed that the highest density of **γ**-terpinene was related to full flowering but the highest densities of thymol and carvacrol were obtained from full flowering and start of fruit ripening. Sefidkon *et al.*, (2009) observed that thymol amount was increased gradually in Garden thyme from the first of vegetative phase to full flowering. Golparvar (2011) in study of Kermanian thyme (*Thymus caramanicus* Jalas.) observed that the lowest essential oil was obtained in vegetative phase. Evaluation of the variations happened in essential oil percentage revealed that this plant has less essential oil in vegetative phase, but after transition to fruit set stage will have obvious increase in essential oil amount that can be because of different external/internal factors. This phenomenon is not only important for essential oil amount but also is interesting from other aspects like changes in amount of some of its components. Environmental factors like temperature, humidity, light, location, soil are important but this is essential to know that clearing up effects of environment will not reduce the role of genetic factors which may be themselves affected by environment (Cristina *et al.*, 2008; Golparvar, 2001; Hudaib and Aburjai, 2007).

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