Phytochemical and Yield Variation among Iranian Achillea millefolium Accessions

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Abstract. Chemical composition and essential oil yields from aerial parts of 31 Iranian Achillea millefolium accessions, each collected from their natural habitats of Iran and grown together in field conditions, were investigated. The concentrations of the hydrodistilled essential oils ranged from 0.03% to 0.39%. Gas chromatography–mass spectrometry (GC–MS) analysis revealed 50 compounds in the accessions. The main components of the essential oils in Iranian A. millefolium accessions varied in the following ranges: 1.8-Cineole, 1.2–19.8%; β-thujone, 0.4–55.3%; camphor, 0.6–25.5%; germacrene-D, 2–20.6%; trans-nerolidol, 0.4–48.1%; isospathulenol, 0.5–36%; and cubenol, 0.1–42.9%. According to cluster analysis, five chemotypes were obtained as 1.8-Cineole/trans-nerolidol, high cubenol, high germacrene-D/isospathulenol, high camphor/cubenol, and high 1.8-Cineole/β-thujone/cubenol. The result of principal component analysis (PCA) indicated that germacrene-D and isospathulenol components were under more genetic control than the other main components. Results revealed a high level of variation in composition and yield of essential oils among the Iranian A. millefolium accessions.

Achillea millefolium L. is one of the most important medicinal plants because of its diverse medicinal uses including anti-infective, spasmytic, choleretic, carminative, and anti-inflammatory activities (Benedek et al., 2007; Naeni et al., 2009). It is spread in many regions of Iran. Investigation about the phytoc hemical components of A. millefolium is important for determining therapeutic agents having potential pharmaceutical value (Farnsworth, 1966; Pandith, 2012).

The many accessions of Iranian A. millefolium are known to produce a wide variety of chemical compounds in the essential oils, with the most abundant components identified as chamazulene, germacrene D, sabine, β-caryophyllene, p-cymene, bornyl acetate, camphene, β-pinene, 1,8-Cineole, camphor, ascaridole, limol, α- and β-thujone, cis- and trans β-ocimene, myrcene, limonene, γ-terpinene, carphyllene oxide, α-bisabolol, β-eudesmol, and α-phellandrene (Dehghan and Elmi, 2015; Ebrahimii et al., 2012; Gudaityte and Venskutonis, 2007; Judzentiene, 2016; Sevindik et al., 2016; Stevanovic et al., 2015). In Iran, most studies on A. millefolium have been focused on only a few accessions that are limited to a single geographical area (Afsharypuor et al., 1996; Jaimand et al., 2006; Maz et al., 2013; Mazandaran et al., 2007). Ebratimi et al. (2012) studied essential oil variation among five Achillea millefolium ssp. ebursensis collected from different ecological regions in Iran. In their study, accessions of sub-species of ebursensis were examined. To the best of our knowledge, there is not a comprehensive study on essential oil components of the plant in Iran.

The objective of this study was to expand the information on the chemical variations in essential oil of A. millefolium accessions from different regions in Iran.

Materials and Methods

Plant materials. Seeds of 37 accessions of A. millefolium were collected from the North-erman, Northwestern, Western, Southern, and central regions of Iran (Table 1) and cultivated in the research farm of College of Abourihan, University of Tehran, in Spring 2012. Voucher samples were deposited at the Herbarium of Research Institute of Forests and Rangelands Tehran, Iran (Farajpour et al., 2012). The randomized complete block design with three replications was used. First, accessions were cultivated in a greenhouse, and then, when the plant reaches to about 10 cm, they were transferred to the filed. Each accession was sowed in 1 m² plots in a sandy-loam soil. The results of soil analysis of the filed are present in Table 2. Accessions were harvested at 10–20% flowering stage to obtain essential oils. For GC-MS analysis, the plants from the three replications were mixed. Finally, for each accession one sample was used to GC-MS analysis. Genetic diversity based on morphological, phenological, and molecular markers from these plants were reported in our previous study (Farajpour et al., 2012).

Isolation of essential oils. Aerial parts of A. millefolium accessions were dried at room temperature. Hundred grams of each sample was extracted by hydro-distillation (1:5 biomass:water ratio) in a 2-L Clever-type apparatus for 3 h. The oils were collected from the top of the apparatus using syringe. Yields of essential oils were calculated based on air-dried materials.

Composition of essential oils. GC-MS analysis was conducted using a Varian CP-3800 GC coupled to a Varian 4000 (Ion trap) mass analyzer. The GC was equipped with a capillary VF-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm), using helium as the carrier gas with constant flow of 1.0 mL min⁻¹ and a split ratio of 1/50. The oven temperature was set at 60 °C for 1 min and then ramped to 250 °C at a rate of 3 °C min⁻¹ where it was held for 10 min. The injector and detector (FID) temperatures were kept at 250 and 280 °C, respectively. Mass spectra were taken at 70 eV with a mass/charge range of 35–400. The chemical composition of the essential oils was determined through calculation of retention indices under temperature-programmed conditions for n-alkanes (C₆–C₂₄) in comparison with the oils on a VF-5 column under the same chromatographic conditions. The compounds were identified by comparison of their mass spectra with those in the Wiley 7 database or with injection of isolated standards. These matches were confirmed by comparison of their retention indices with that of known compounds or with those reported in the literature. For quantification purposes, relative area percentages obtained by FID were used without any correction factor.

Statistical analysis. Cluster and PCA were performed using SPSS v.23, and calculation of correlations was performed using statistical analysis system (SAS) computer software (SAS institute Cary, NC, 1988). In addition, the means scanning electron microscopy of the oils’ content were compared.
using least significant difference test (Steel and Torrie, 1980).

Result and Discussion

The essential oils yield. The essential oils yield of *A. millefolium* (mean values from three replications) varied in the range from 0.03% to 0.39% (Table 1). The highest content of essential oils was found in accession collected from Arak, Markazi Province (Am1 accession); it was 0.39%. The lowest amount of essential oil, 0.03%, was distilled from two accessions collected from Fars Province (Am5 and Am17 accessions). The authors found the yields of essential oil in the range of 0.15–0.60%. This result suggests that environmental factors play an important role in the quantity of essential oil yields in Iranian *A. millefolium* accessions as their plants were grown under different time and geographical conditions. However, our accessions had higher essential oil content than Lithuanian *A. millefolium* samples (in the range of 0.06–0.19%; Gudaityte and Venskutonis, 2007) because of using many accessions from different habitats.

Furthermore, these results showed no obvious relationship between the location of accession and essential oil yield. For example, Am1, Am2, and Am3 with high essential oil yields were collected from the center, north, and west of the country, respectively.

Essential oil composition. The composition of each essential oil of the 31 Iranian *A. millefolium* accessions was analyzed by GC-MS (Table 3). A total number of 50 compounds were identified across all accessions. Forty-two of these compounds were observed in Am13 accession. However, 117 compounds were identified in Lithuanian *A. millefolium* samples (Gudaityte and Venskutonis, 2007) because of using many accessions from different habitats. All the accessions had 1,8-Cineole and camphor components. cubenol was the most abundant component in seven essential oil accessions, Am15, Am16, and Am17 accessions were rich in limonene (15%); Am6 and Am29 accessions were rich in pino-camphor (18.7% and 15.8%, respectively); Am15, Am16, and Am17 accessions were rich in borneol (10.6%, 10.5%, and 17.8%, respectively); Am3 accession was rich in carvone (18.7%) in 0.15–0.60%. This result suggests that environmental factors play an important role in the quantity of essential oil yields in Iranian *A. millefolium* accessions as their plants were grown under different time and geographical conditions. However, our accessions had higher essential oil content than Lithuanian *A. millefolium* samples (in the range of 0.06–0.19%; Gudaityte and Venskutonis, 2007) because of using many accessions from different habitats.

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two accessions, and isospathulenol dominated in one accession. 9-epi-(E)-Caryophyllene was observed only in one accession (Am13). Additional unique components in each accessions are shown in Table 3. Dokhani et al. (2005) identified the major compounds of some selected *Achillea* species in Iran. High interspecies and intraspecies polymorphisms were observed in the studies. In our study, cubenol was the most abundant component. 1,8-Cineole, camphor, terpinolene, borneol, γ-terpinene, and thujone were the major components of the essential oils in *A. millefolium* from different regions in North east of Iran (Maz et al., 2013). In the present study, four accessions were from north east of Iran, 1,8-Cineole and camphor were just two of the seven main components previously reported. Orav et al. (2007) studied phytochemical analysis of the essential oil of *A. millefolium* L. from various European countries. Authors indicated that quantitatively the most important components of the plant were bornyl acetate, 1,8-Cineole, sabine, artemisia ketone, camphor, β-pinene, linalol, caryophyllene oxide, α-thujone, β-thujone, β-bisabolol, borneol, fenchyl acetate, (E)-β-caryophyllene, germacrene D, δ-cadinol, and chamazulene. According to literature reviews, there were many different reports about the main essential oil components in *A. millefolium* samples. Information about the factors that control the chemical variability and yield for each medicinal plant is very important but highly understudied. These include physiological variations, environmental factors, geographic variations, genetic factors and evolution, social conditions, and the amount of plant material (Figueredo et al., 2008).

*A. millefolium* chemotypes based on their chief components. The cluster analysis was performed using the seven identified main components. From this analysis, the Iranian *A. millefolium* accessions were categorized into five groups (Fig. 1). The first group contained of ten accessions (Am25, Am27, Am7, Am15, Am3, Am31, Am9, Am24, and Am19). The accessions of the first group were rich in 1,8-Cineole and trans-nerolidol. Am19 accession of this group was separated from others because of the highest percentage being rich in both camphor and cubenol. In

### Table 3. Chemical composition of essential oils (%) of 31 Iranian *Achillea millefolium* accessions.a

| Component | RI | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|-----------|--------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1,8-Cineole | 1,036 | 2.28 | 7.43 | 15.6 | 23.8 | 6.32 | 2.94 | 13.81 | 3.02 | 19.83 | 8.77 | 7.88 | 1.22 | 7.19 | 16.1 | 13.3 | 13.5 |
| δ-Terpine | 1,061 | 49.39 | | | | | | | | | | | | | | | | |
| β-Thujone | 1,112 | 28.97 | 5.84 | 4.16 | 4.90 | 1.05 | | | | | | | | | | | |
| trans-Verbenol | 1,151 | 11.91 | 2.03 | 2.86 | 3.94 | 4.01 | 2.71 | | | | | | | | | | |
| Camphor | 1,154 | 7.88 | 6.01 | 19.4 | 2.60 | 25.50 | 7.77 | 1.30 | 10.20 | 6.19 | 3.69 | 3.27 | 3.13 | 6.83 | 2.24 | 3.18 |
| Pinocarvone | 1,168 | | | | | | | | | | | | | | | | |
| Borneol | 1,178 | | | | | | | | | | | | | | | | |
| cis-ChrysanthenylAcetate | 1,260 | 0.65 | 5.64 | 1.30 | | | | | | | | | | | | |
| Bornyl acetate | 1,288 | | | | | | | | | | | | | | | | |
| Caryophyllene | 1,425 | | | | | | | | | | | | | | | | |
| Germacrene-D | 1,487 | 12.71 | 4.80 | 10.5 | 8.28 | 8.03 | 8.01 | 1.98 | 4.75 | 4.90 | 7.47 | 20.64 | 4.83 | 10.8 | 12.1 | 3.26 |
| α-Murolene | 1,501 | 1.17 | | | | | | | | | | | | | | | |
| Calacorene | 1,530 | 25.08 | 2.68 | 2.40 | 1.09 | 2.24 | 1.59 | | | | | | | | | |
| trans-Nerolidol | 1,570 | 25.1 | | | | | | | | | | | | | | | |
| Isospathulenol | 1,592 | 1.94 | 6.01 | 5.04 | 2.32 | 5.92 | 7.98 | 4.51 | 5.03 | 5.05 | 36.00 | 4.27 | 5.40 | 2.76 | 6.64 |
| Caryophyllene oxide | 1,598 | 0.76 | 1.00 | 11.9 | 2.01 | 6.90 | 1.94 | 0.94 | 0.47 | 8.51 | 3.02 | 2.65 | 8.32 | 3.37 | | |
| Cubenol | 1,648 | 12.71 | 10.8 | 36.24 | 10.88 | 9.71 | 37.77 | 10.73 | 30.72 | 30.49 | 0.03 | 9.39 | 3.14 | 22.6 |
| β-Eudesmol | 1,667 | 1.14 | 1.23 | 4.90 | 1.81 | 10.78 | 0.75 | 5.42 | 1.15 | 0.76 | | | | | |

The major compounds are presented as bold.

### Table 4. Principal components databased on 7 major oil compounds of 31 Iranian *Achillea millefolium* accessions.

| Label | Major compound | PC1 | PC2 | PC3 |
|-------|----------------|-----|-----|-----|
| 1     | Germacrene-D   | 0.9 | 0.08 | 0.25 |
| 2     | Isospathulenol  | 0.71 | 0.49 | 0.09 |
| 3     | 1,8-Cineole    | -0.19 | 0.75 | -0.09 |
| 4     | Trans-nerolidol| 0.28 | 0.69 | -0.42 |
| 5     | Cubenol        | -0.42 | -0.60 | -0.45 |
| 6     | Beta-thujone   | -0.49 | 0.03 | 0.60 |
| 7     | Camphor        | -0.10 | 0.15 | 0.59 |

The values higher than 0.5 are presented as bold significant.

The third group consisted of Am21, Am26, Am1, Am17, and Am12 accessions. The main components of this group were germacrene-D and isospathulenol. Many unique components were found in the group: Am26 and Am1 accessions were rich in γ-terpinene and calacorene, Am21 accession was rich in β-eudesmol and torreyol, and Am17 accession was rich in limonene and borneol. Am4 and Am6 were the only two accessions classified in the fourth group, being rich in both camphor and cubenol. In
our previous study on genetic variation among the 31 accessions using inter-simple sequence repeat (ISSR) marker, these two accessions were classified at cluster IV (Farajpour et al., 2012), both of them were gathered from the western part of the country. The fifth group comprised ten accessions. The mean percentage of β-thujone in this group was higher than that of the other groups; however, the group was rich in 1,8-Cineole and cubenol. Am23 had the highest percentage of β-thujone (55.3%).

According to cluster analysis and the concentrations of the seven main compounds in each cluster, the essential oils of Iranian Achillea millefolium accessions were classified into five chemotypes:

1) 1,8-Cineole (13.4%) + transnerolidol (16.1%; 10 accessions)
2) Cubenol (35.6%; 5 accessions)
3) germacrene-D (10.7%) + isospathulenol (12.5%; 5 accessions)
4) Camphor (22.5%) + cubenol (10.8%; 2 accessions)
5) 1,8-Cineole (9.2%) + β-thujone (16.9%) + cubenol (10.8%; 9 accession)

PCA was achieved for the seven main compounds in the essential oils of Iranian Achillea millefolium accessions. The first three principal components (PCs) confirmed 67.8% of the total variance (Table 4). The first PC confirmed for 27.3% of the total variance and correlated positively with germacrene-D (0.9) and isospathulenol (0.71). 1,8-Cineole (0.75) and transnerolidol (0.69) components showed a positive, and cubenol (-0.6) indicated a negative correlation but with the second PC. The third PC confirmed more than 16% of the total variance and correlated positively with β-thujone (0.6) and camphor (0.59). Because all accessions were cultivated in the same location, the main contributor to variation among the accessions is genetic factors. Thus, it seems the first factor (PC1) is a genetic factor. The PC1 was highly correlated with germacrene-D and isospathulenol; hence, the two components are under more genetic control than the other components.

**Conclusion**

Herein, a comprehensive phytochemical analysis of essential oils among Iranian Achillea millefolium accessions was performed. The result of the present study showed that there was a high phytochemical and essential oil variation among 31 Iranian Achillea millefolium accessions. The main components among the studied accessions were 1,8-Cineole, 1.2–19.8%; β-thujone, 0.6–55.3%; camphor, 0.6–25.5%; germacrene-D, 2–20.6%; transnerolidol, 0.4–48.1%; isospathulenol, 0.5–36%; and cubenol, 0.1–42.9%. The result revealed five chemotypes in Iranian Achillea millefolium accessions according to main compounds including high 1,8-Cineole/transnerolidol, high cubenol, high germacrene-D/isospathulenol, high camphor/cubenol, and high 1,8-Cineole/β-thujone/cubenol.

**Literature Cited**

Afsharypour, S., S. Asgary, and G.B. Lockwood. 1996. Volatile constituents of Achillea millefolium L. ssp. millefolium from Iran. Flavour Fragrance J. 11(5):265–267.

Anwar, F. 2009. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel (Foeniculum vulgare Mill.) fruit at different stages of maturity. J. Herbs Spices Med. Plants 15:1–16.

Benedek, B., B. Kopp, and M.F. Melizg. 2007. Achillea millefolium L. ssp. elbursensis Hub.-Mor. from Iran rich in chamazulene. J. Essent. Oil Res. 18(3):293–295.

Judzentiene, A. 2016. Atypical chemical profiles of wild yarrow (Achillea millefolium L.). essential oils. Rec. Nat. Prod. 10(2):262–268.

Maz, M., S.Z. Mirdeilami, and M. Pessarakli. 2013. Essential oil composition and antibacterial activity of Achillea millefolium L. from different regions in North east of Iran. J. Med. Plants Res. 7(16):1063–1069.

Mazandarani, M., B. Behmanesh, M.B. Rezaei, and E.O. Ghaemi. 2007. Ecological factors, chemical composition and antibacterial activity of the essential oil from Achillea millefolium L. in the north of Iran. Planta Med. 73(9):P-179.

Orav, A., E. Arak, and A. Raal. 2006. Phytochemical analysis of the essential oil of Achillea millefolium L. from various European Countries. Nat. Prod. Res. 20(12):1082–1088.

Naeini, A., A.R. Khosravi, M. Chitsaz, H. Shokri, and M. Kamlehydrad. 2009. Anti-Candida albicans activity of some Iranian plants used in traditional medicine. J. Med. Mycol. 19(3):166–172.

Pandith, J.I. 2012. Phytochemical screening of certain plant species of Agra City. J. Drug Deliv. Ther. 2(4):135–138.

Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill, Inc., NY.

Sevendidik, E., Z.T. Abaci, C. Yamaner, and M. Ayvaz. 2016. Determination of the chemical composition and antimicrobial activity of the essential oils of Teucrium polium and Achillea millefolium grown under North Anatolian ecological conditions. Biotechnol. Biotechnol. Equip. 30(2):375–380.

Stevanovic, Z.D., D. Pjevljakusic, M. Ristic, I. Soštaric, M. Kresovic, I. Simic, and S. Vrbićanen. 2015. Essential oil composition of Achillea millefolium agg. populations collected from saline habitats in Serbia. J. Essent. Oil Bear. Plants 18(6):1343–1352.