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Research Article

THE EFFECTS OF GROWTH HABITAT OF MEDICINAL PLANT YARROW (*ACHILLEA WILHELMSII* C.KOCH) ON THE ESSENTIAL OIL CONSTITUENTS

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ABSTRACT

It is known that growth conditions can affect the amount and types of the essential oil constituents of many plants. In the present study, aerial parts of *Achillea wilhelmsii* at the flowering stage were collected from seven locations of Dena and Boyer-Ahmad, located in Kohgiluyeh and Boyer-Ahmad province (latitude 30.67 N, longitude 51.60E), and their essential oils were extracted by means of steam distillation. The chemical constituents of the essential oils were analyzed using GC/MS. The results showed that the amounts of essential oils varied from 0.1 up to 0.56 ml per 100 gr DW of the plant species. Variations were also observed in the constituents of the essential oils. The ten main compounds were 1,8-cineol, linalool, E-nerolidol, caryophyllene oxide, α-pinene, α-terpineol, thymol, terpinene-4-ol, camphor and P-cymene. It is concluded that altitude, together with different environmental conditions, could affect the degree of biosynthesis and accumulation of essential oils in Achillea wilhelmsii.

Keywords: *Achillea Wilhelmsii, Altitude, Essential Oils*

INTRODUCTION

Achillea wilhelmsii C.Koch is a perennial medicinal herb belonging to the family of Compositae (Asteraceae), which comprises more than 120 species. The plants are native to Europe and western Asia, though populations have also been discovered in Australia, New Zealand, and North America. Nineteen species of the genus Achillea have been recorded in the Flora Iranica. *Achillea wilhelmsii* C.Koch, locally known as "boomadaran", is the major species which grows wildly in some regions of Iran and is widely used in Iranian traditional medicine (Rechinger, 1963; Mozaffarian, 1966; Dokhani *et al.*, 2005).

Several pharmaceuticalproperties of Achillea, such as its anti-inflammatory (Candan *et al.*, 2003), antibacterial (Candan *et al.*, 2003; Stojanovic *et al.*, 2005; Mahady *et al.*, 2005), antitumor (Tozyo *et al.*, 1994; Csupor-Loffler *et al.*, 2009), antispasmodic (Lemmens-Gruber *et al.*, 2006; Yaeesh *et al.*, 2006), choleretic (Benedek *et al.*, 2006), antiulcer (Cavalcanti *et al.*, 2006), and antiallergic (Asgary *et al.*, 2000) characteristics, have been documented. The cosmetic and healthcare industries make use of A. wilhelmsii essential oils to produce skin ointment and to treat skin inflammations (Pieroni *et al.*, 2004). Some studies have indicated that Achillea species such as A. santolina (Ardestani and Yazdanparast, 2007), A. ligustica (Tuberoso *et al.*, 2005) and A. clavennae (Stojanovic *et al.*, 2005) have antioxidant properties which can inactivate free radicals. It has also been shown that Achillea contains aromatic bitter substances and tannins which have noticeable effects on the nervous system and are effective in the treatment of neurological diseases such as neurasthenia, epilepsy and seizures (Azadbakht *et al.*, 2003).

The genus Achillea is rich in flavonoids, sesquiterpene lactones, and monoterpenoids, which have antioxidant activity (Baris *et al.*, 2006; Candan *et al.*, 2003; Nemeth and Bernath, 2008) and also have been shown to be effective in lowering both blood lipids and hypertension (Asgary *et al.*, 2000). Its major chemical components are alkaloids (achilleine), cineol, borneol, α- and β-pinene, luteolin, apigenin, lignans, camphor, caryophllene, thujene, rutin and carvacrol (Dokhani *et al.*, 2005; Afshrypour *et al.*, 1996; Javidnia *et al.*, 2004; Jaimand and Rezaee, 2001).

Achillea wilhelmsii growing in different parts of Iran such as Fars (Javidnia *et al.*, 2004), Mazandaran (Azadbakht, 2003) and Kerman (Afshrypour *et al.*, 1996) provinces have been analyzed for both their chemical compositions and essential oils. However, no such reports are available for *Achillea wilhelmsii* growing in different parts of Kohgiluyeh and Boyer-Ahmad province, which is an important geographical region for medicinal plants.

In the present study, the essential oil compositions of *Achillea wilhelmsii* C.Koch inflorescence collected from seven localities throughout the Boyer-Ahmad and Dena region in Kohgiluyeh and Boyer-Ahmad province were investigated, with the results indicating that changes in essential oil yields and compositions in this important medicinal plant depend mainly on climatological and ecological conditions.

It is known that the essential oil yield and chemical composition of this species of plants can vary considerably depending on the edaphoclimatic conditions of the area in which it grows. For this reason, the oil from samples of Achillea wilhelmsii C.Koch growing at different altitudes in Kohgiluyeh and Boyer-Ahmad province were analyzed via GC-GC/MS

MATERIALS AND METHODS

Plant Material

Samples of the aerial parts of *Achillea wilhelmsii* C. Koch, namely the stems and flowers, at flowering stage were collected in June 2013 from seven locations with differing altitudes. Four of these samples from obtained from the Dena region of Iran. These included sample **A** from Padena (2448m), sample **B** from Amirabad (1890m), sample **C** from Bhrambegi (1800), and sample **D** from Kara (1739m). The other three samples were collected from the Boyer-Ahmad region of Kohgiluyeh and Boyer-Ahmad province in Iran, including sample **E** from Galal (2373m), sample **F** from Thlion (1793m), and sample **G** from Chin (1485m). The plants were taxonomically identified by our botanist at the herbarium of the Biology Department of Shiraz University in Iran.

Essential Oil Extraction

The aerial parts of the plants were air dried at room temperature (20-25°C) in the dark. The essential oils were extracted by means of the hydrodistallation of 50gr of each sample for three hours using a Clevenger-type apparatus. The obtained essential oils were then dried over anhydrous sodium and stored in sealed vials at 4°C in the dark until analyzed.

Identification of the Essential oil Constituents

The analysis of the essential oils was carried out by GC and GC/MS. GC analysis was carried out using an Agilent-technology chromatograph with a HP-5 column (30m × 0.32mm i.d ×0.25 µm). Oven temperature was set as follows: 60˚C to 210˚C at 3˚/min; 210˚C to 240˚C at 20˚/min and hold for 8.5 min, injector temperature 280˚C; detector temperature 290˚C; carrier gas N2 (1 ml/min); split ratio of 1:50. GC-MS analysis was carried out using an Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30m × 0.25 mm i.d × 25µm), with helium as the carrier gas and a spilt ratio of 1:50. Retention indices were determined using the retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all constituents were determined according to the method using n-alkanes as standard. The compounds were identified by comparing the retention indices (RRI, HP-5) with those reported in the literature and via comparison of their mass spectra with Adams Library and stored in NIST and Wiley Libraries (Mclafferty and Stauffer, 1989; Joulain *et al.*, 2001; Adams, 2007).

RESULTS AND DISCUSSION

The yields of essential oils of *Achillea wilhelmsii* C. Koch collected from different regions in Kohgiluyeh and Boyer-Ahmad province and extracted by the hydrodistillation of the finely powdered flowers were calculated on a 100 gr dry weight basis as 0.44, 0.42, 0.54, 0.54, 0.56, 0.36 and 0.1 milliliters from A to G, respectively. The chemical compositions of the oils are presented in **Table 1**. The main component of the oils in samples A, E and G was 1, 8-cineol (25.62%, 28.52 and 36.98%, respectively). 1,8-cineol has also been reported as the major constituent of the oil of A. wilhelmsii from Egypt, Turkey and

Mazandaran in Iran (Brunke *et al.*, 1986, Azadbakht *et al.*, 2003) and some other Achillea species (Rustaiyan *et al.*, 1999, Weyerstahl *et al.*, 1997, Shawl *et al.*, 2002, Tasic *et al.*, 1998).

Table 1: Chemical composition (%) of the essential oils of *Achillea wilhelmsii* C. Koch growing in regions with different altitudes

		Dena regions				Boyer-Ahmad regions		
Compounds	**RI***	**A (2448m)**	**B (1890m)**	**C (1800m)**	**D (1739m)**	**E (2373m)**	**F (1793)**	**G (1485m)**
Tricyclene	923	-	-	0.04	-	-	-	-
α-Thujene	926	0.12	-	0.06	0.08	0.15	0.10	0.30
α-Pinene	936	5.94	10.11	4.21	6.88	5.72	4.76	4.06
Benzaldehyde	962	-	0.13	0.09	0.09	-	0.10	0.22
Camphene	945	0.13	1.05	0.11	0.54	0.40	0.23	-
Sabinene	975	5.12	0.92	0.52	0.06	3.74	0.75	3.73
β-Pinene	978	1.38	1.18	0.21	0.72	1.39	0.60	1.00
Myrcene	997	0.05	2.58	-	-	1.23	1.19	-
n-Decane	1003	1.92	-	0.06	-	-	0.16	-
α-Phellandrene	1005	0.40	-	0.17	-	-	-	-
α-Terpinere	1017	1.85	1.55	0.53	2.05	-	0.81	1.60
P-Cymene	1026	0.94	0.41	1.59	1.88	1.16	1.86	2.09
Limonene	1031	1.08	0.33	0.80	0.32	1.12	0.28	3.76
1,8-Cineole	1034	25.62	3.65	3.09	4.60	28.52	9.50	36.98
(E)-β-Ocimene	1052	0.11	-	0.09	-	0.08	-	0.10
Terpinene	1062	0.49	-	0.13	4.32	0.58	4.17	1.67
cis-Sabinene hydrate	1069	0.41	-	0.21	7.64	0.67	6.88	1.55
Trans-Linalool oxide	1076	-	-	-	-	-	0.23	-
Terpinolene	1089	0.09	-	0.15	0.91	0.09	0.96	0.35
Linalool	1100	21.48	13.31	2.17	7.40	14.20	19.00	1.87
n-Nonanal	1107	-	-	0.81	-	-	-	1.13
n-Amyl isovalerate	1112	0.95	0.58	0.19	0.41	0.67	1.22	0.68
cis-P-Menth-2-en-1-ol	1124	-	-	7.00	0.73	0.90	0.83	0.29
α-Campholenal	1129	0.14	0.24	-	0.24	0.44	0.37	1.26
Chrysanthenone	1131	-	0.36	1.12	-	-	-	-
trans-P-Menth-2-2n-1-ol	1141	-	-	5.01	0.66	0.89	0.83	1.07
Trans-Pinocarveol	1142	0.16	-	-	-	-	-	-

Camphor	1149	0.20	2.21	0.85	0.56	1.19	0.82	0.31
Pinocarvone	1160	1.37	1.69	1.28	0.55	1.96	1.58	2.79
cis-Chrysanthenol	1165	-	-	11.73	-	-	-	-
Borneol	1166	0.21	1.70	-	0.44	1.61	0.71	0.44
Terpinene-4-ol	1177	0.62	0.21	0.55	10.33	2.41	10.88	3.39
α-Thujenol	1181	0.89	-	0.51	-	1.66	-	-
α-Terpineol	1197	3.45	0.87	2.27	0.69	4.51	1.62	4.11
n-Dodecane	1201	0.08	0.58	-	0.61	0.42	0.35	0.36
trans-Piperitol	1211	-	-	8.45	-	0.76	-	0.16
cis-Carveol	1229	-	-	0.19	-	-	-	0.37
Neo iso-DihydroCarveol	1234	0.42	-	-	-	0.35	0.11	0.17
Cis-Chrysanthenyl acetate	1263	-	-	33.47	-	3.63	-	-
Thymol	1292	1.12	0.74	0.43	33.48	0.34	13.11	6.58
Carvacrol	1309	-	-	-	0.27	-	0.13	0.18
δ-elemene	1335	-	0.20	-	-	-	-	-
Eugenol	1360	0.12	-	-	-	0.14	-	0.17
Geranyl acetate	1384	-	-	-	0.16	0.20	0.12	0.80
(z)-Jasmone	1401	0.14	0.30	2.13	0.15	0.24	0.11	0.20
(E)-Caryophyllene	1419	0.34	0.93	0.15	0.16	0.37	0.29	1.16
Bicyclogermacrene	1496	0.27	1.47	2.79	-	-	0.13	0.19
(E)-Nerolidol	1562	12.26	18.59	2.79	1.16	10.09	2.43	0.17
Spathulenol	1578	0.30	0.55	0.08	0.59	0.45	0.55	0.98
Caryophyllene oxide	1588	1.43	3.57	01.33	3.75	2.13	2.64	5.47
Caryophylla-4(14),8(5)-dien-5-β-ol	1638	0.24	0.64	0.54	1.29	0.51	1.11	2.07
α-Muurolol	1642	-	0.52	-	-	-	-	-
β-Eudesmol	1653	0.59	1.41	0.79	0.33	0.58	0.58	0.52
α-Cadinol	1658	0.31	-	-	-	-	-	0.24
(2E,6E)-Farnesol	1721	0.17	0.34	0.78	-	-	-	-
epi-α-Bisabolol acetate	1810	0.44	1.13	0.23	0.42	0.53	0.60	-
Cyclopentadecanolide	1834	-	0.40	-	-	-	-	-
(3E)-Cembrene A	1950	5.27	22.42	-	1.79	2.85	3.42	-
Unknown		1.38	3.13	0.3	3.74	1.12	3.88	5.46

RI: Retention Index

In the analysis of the essential oil of sample B, (E)-neurolidol (18.59%), linalool (13.31%) and α-pinene (10.11%) were found to be the next most important constituents. As regards sample C, the six major consituents were cis-chrysanthenyl acetate (33.47%), cis-chrysanthenol (11.73%), trans-piperitol (8.45%), cis-P-Ment-2-en-1-ol (7%), α-pinene (4.21%) and 1,8-cineole (3.09%). Moving on, the primary constituents of samples D, B and F were thymol (33.48%), (3E)-cembrene A (22.42%) and linalool (19%), respectively. The amounts of thymol and linalool in the A. wilhelmsii oil sample collected from Golmakan, KorasanRazavi were 5.28% and 12%, respectively (Hoseini-Alfatemi *et al.*, 2014). Samples A and E rich in 1,8-cineol contained large proportions of linalool (21.48% and 14.20%, respectively) and (E)-nerolidol (12.26%and 10.09%, respectively), making them the other major constituents of these samples, followed by and α-pinene (~ 5%). Of the 36 compounds identified in the essential oil of A. wilhelmsii sample D with 96.26%, the combination of thymol (33.48%), terpinene-4-ol (10.33%), cis-sabinene hydrate (7.64%), linalool (7.40%), α-pinene (6.88%) and 1,8-cineol (4.60%),at 70.33 percent, constitute the highest percentage of this essential oil. Nineteen components in the volatile oil of A. wilhelmsii from Kerman (Iran) were identified and reported previously, of which caryophyllene oxide (12.5%), camphor (9%), borneol (6.1%), linalool (5.5%), 1,8-cineol (3.6%) were the main elements (Adams, 2007). The combination of 1,8-cineol (36.98%), thymol (6.58%), caryophyllene oxide (5.47%), α-pinene (4.06%), α-terpineol (4.11%), limonene (3.76%), and sabinene (3.73%), at 64.69 percent, constitute the highest percentage of the essential oil of sample G. Regarding the constituents of the essential oils obtained from sample F, linalool accounted for the largest proportion (19%), followed bythymol (13.11%), terpinene-4-ol (10.88%), 1,8-cineol (9.5%), and cis-sabinene hydrate (6.88%). The main components of the A. wilhelmsii oil from Kazeroon in Fars province were carvacrol (25.1%), linalool (11%), 1,8-cineol (10.3%), E-nerolidol (9%) and borneol (6.4%) (Javidnia *et al.*, 2004). Overall, a total of 22 compounds were present in all of the samples; however, there were also several compounds that were found only in certain samples. Trans-pinocarveol was detected only in sample A. δ-eleme,α-muurolol, and cyclopentadcanolid were present only in sample B, whilecis-chrysanthenol was found in sample C alone. In samples A, B, C, and Dcollected from Dena, the amount of sabinene decreased hand in hand with falling altitudes, whereas (2E.6E)-farnesol increased as altitude rose. With respect to samples E,F, and G obtained from Boyer-Ahmad, the lower the altitude was, the lower the proportions of the following constituents were: α-pinene, Cis-P-menth-2-en-1-ol, camphor and (E)-nerolidol. By contrast, larger quantities of P-cymene and caryophyllene oxide were typically associated with lower altitudes. These qualitative and quantitative variations in the composition of the essential oils from different locations in Iran, or from other countries for that matter, are likely the result of genetic variation, growth conditions, geographic variation and analytical protocols used to assess the essential oils.

The chemical compositions of the A.wilhelmsii C. Koch essential oils from different areas in Kohgiluyeh and Boyer-Ahmad province varied markedly and, in the case of some of the major components, were close to those of the plants which grow in other provinces. Due to the presence of valuable compounds, A. wilhelmsii is emerging as one of the most important medicinal plants in many parts of the world.

Conclusion

The present study shows that environmental conditions such a altitude have profound effects on amounts and chemical composition of Achillea willhelmsii essential oils collected from different regions of Kohgiluyeh and Boyer-Ahmad province, Iran. The amount of essential oil ranged from 0.04% to 33.48% and number of chemical compounds present in the oil ranged from 36 to 44.

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