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Essential Oil Composition of *Achillea aucheri* Boiss at Different Growing Altitudes in Damavand, Iran

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ABSTRACT

The genus *Achillea* is one of the most important medicinal plants in the Asteraceae family. Nineteen species of this genus have been described in the Flora Iranica, of which *Achillea aucheri* Boiss is an endemic species to Iran. Given the effect of geographic location on the quality and yield of essential oil, we performed an investigation to determine oil contents of *A. aucheri* growing at different altitudes of Mount Damavand and analyzed its components. Essential oil was extracted with hydro-distillation method and nearly 96.20% of oil components were identified using GC and GC/MS, on average. These components included isopulegol (16.39%), yomogi alcohol (10.92%), (Z)-*β*-ocimene (9.84%), camphor (5.65%), 1,8-cineole (4.98%), linalool (4.81%), *γ*-terpinolene (3.66%), *α*-fenchene (3.60%), camphene (3.20%), *α*-pinene (3.15%), *α*-terpineol (2.71%), nerolidol (2.54%), chamazulene (1.74%) and *α*-thujone (1.67%), with 34 other components. In general, essential oil composition of *A. aucheri* was influenced by climatic condition prevailing at different altitudes. By increasing altitude, constituent of α-thujone, a harmful compound for human body, decreased in its value. Moreover, going from 3,900 to 4,300 m altitude, the valuable constituent of chamazulene increased more than 10 times.

Keywords: *Achillea* genus, Chemotype, Climate condition, Endemic species, Medicinal plant.

INTRODUCTION

Essential oils are complex mixture and constitute terpenoid hydrocarbons and oxygenated terpenoids. They originate from plant secondary metabolism and are responsible for their aromatic characteristics (Figueiredo *et al.,* 2008). The genus *Achillea* is one of the most important medicinal plants in the Asteraceae family (Nemeth, 2005). Nineteen species of this genus have been described in the Flora Iranica, of which *Achillea aucheri* Boiss is one of the most important endemic species to Iran (Podlech *et al.,* 1986). It grows naturally in spring and practically disappears at the beginning of winter, developing its complete life cycle within this time span. The plants in genus *Achillea* have been known for many years and their dried aerial parts with flowering tops are used in folk medicine (British Pharmacopoeia Commission, 2009). They help regulation of the menstrual cycle and reduce heavy bleeding and pain. It also has been proposed to be used as an antibiotic alternative in broiler chickens (Yakhkeshi, *et al.,* 2012).

Severity of environment associated with increasing altitude in mountain ecosystems can affect medicinal plants growth as well as their chemical compositions. These variations might be due to the presence of different chemotypes, plants adaptation to the surrounding environment, and developmental stage. Increasing altitude resulted in a decrease in relative and

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absolute allocation of biomass to reproductive structures of weed *Achillea millefolium* (Johnston and Pickering, 2004). Essential oil of *A. aucheri* and *A. kellalensis* showed different patterns in their composition even though they were collected at the same developmental stage but from different geographical regions of Iran (Rustaiyan *et al.,* 1999).

Giorgi *et al.* (2005) reported that *α*- and *β*-thujone were not present in oils of *Achillea millefolium* L. from fields at a higher altitude. They proposed the influence of both genetic and environmental factors on oil synthesis and composition. Leaf extracts of *Achillea collina* from the higher altitude showed a 2-4 fold increase of chlorogenic acid level (Giorgi *et al.,* 2010). Nchabeleng *et al*. (2012) reported a positive correlation (R^2= 0.55) between total polyphenol content and altitude in wild bush tea. Polyphenol content reached a maximum of 7.7 mg 100 g^{-1} in bush tea samples from Haenertsburg region at altitude of 1,410 m, whereas the lowest content of 3.6 mg 100 g^{-1} was recorded in Levubu at altitude of 671 m. They mentioned that high polyphenol content of plants from Haenertsburg region was mainly due to high altitude of this area. Khan *et al.* (2010), showed the importance of habitat characteristics in the biosynthesis of phyllanthin in *Phyllanthus amarus* plant. Among 23 studied populations, only 16 populations showed increased phyllanthin content with elevated altitude. The relationship between environmental and genetic factors and oil composition in some *Achillea* species has also been investigated (Dokhani *et al.,* 2005; Rahimmalek *et al.,* 2009).

Given that the applications properties of essential oil are based on their composition, presence or absence of specific components give certain criteria to the oils. All these facts suggest that a deep study of the native *A. aucheri* oils is necessary at the regional level. Hence, in current research, we aimed at identifying and quantifying the essential oil components of *A. aucheri* in terms of different growing altitudes in Mount Damavand.

MATERIALS AND METHODS

Plant Materials

Aerial parts of *A. aucheri* were collected in July, during the flowering stage, which is the time habitually used for essential oil extraction. Around 6-8 plant samples of 2-3 years old from each altitude were collected from Mount Damavand located at east of Tehran Province, at altitudes of 3,900 upto 4,300 meters. The plant was identified by the Department of Botany of Forests, Range and Watershed Management Organization and a voucher specimen (AC-102975) has been deposited over there.

Extraction of the Essential Oil

Aerial parts of *A. aucheri* from each altitude were pooled together and dried at room temperature, then, grossly pulverized plant powder (100 g) were hydro-distilled using a Clevenger type apparatus for 4 hours. Oil was dried over anhydrous sodium sulfate and, after filtration, stored at +4°C until tested and analyzed.

Gas Chromatography

GC analysis was performed on a Thermo-UFM (Ultra-Fast Model) gas chromatograph equipped with a flame ionization detector. Helium gas was used as the carrier (at a flow rate of 0.7 mL min^{-1}) through the capillary column model Ph-5. The GC oven temperature was set at 60°C for 3 minutes, then programmed at a rate of 5 °C min^{-1} to 80°C, held for 3 minutes, then, programmed from 160 to 230°C at a rate of 10 °C min^{-1} and finally isothermal at 230°C for 12 minutes. The

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injector and detector temperatures were 230 and 250°C, respectively.

Gas Chromatography–Mass Spectrometry

Analysis of the essential oil was performed using an Agilent 7000, Triple Quad, GC 7890A, equipped with a HP-5 MS capillary column (30 m×0.25 mm, id= 0.25 µm) and a HP 5972 mass selective detector. For GC-MS detection, an electron ionization system was used with ionization energy of 70 eV. Helium was the carrier gas, at a flow rate of 1 mL min^{-1}. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially set at 50°C, and then gradually increased to 150°C at a 3 °C min^{-1} rate, held for 10 minutes and finally increased to 250°C at 10 °C min^{-1}. The components were identified based on the comparison of their relative retention time and mass spectra with those of NIST library data of the GC-MS system, literature data and standards of the main components. The results were also confirmed by the comparison of the compounds with their relative retention indices in the literature (Adams, 2001).

RESULTS AND DISCUSSION

Due to its temperate climate conditions, Damavand region in Tehran Province is a good candidate area for growing *A. aucheri* Boiss. A picture of this plant accession, endemic to Damavand region, has been shown in Figure 1.

Figure 1. A bush of *Achillea aucheri* growing at altitude of 4000 m in Mount Damavand , Tehran, Iran.

As a starting point for the study of essential oil, it is necessary to define the influence of plant growing environmental conditions on oil yields and its components. The geographical coordinates of different growing altitudes and the variations in essential oil content of *A. aucheri* are shown in Table 1. Based on the results, the oil percentage increased significantly at high altitudes and varied from 0.68 to 0.95% at the lowest and highest altitudes, respectively. Abad *et al.* (2012) highlighted the effect of geographic location on the quality and yield of essential oil from *Artemisia* species. The oil percent of *Artemisia roxburghiana* was lowest (0.2%) in the plants collected from the relatively higher altitude compared to those of lower ones (Haider *et al.,* 2009).

The essential oil of *A. aucheri* Boiss, blue in colour, was subjected to GC and GC/MS analysis and its constituents were identified by Kovats Retention Index (RI) and mass spectra (Table 2). Nearly 96.20% of oil

Table 1. Geographical coordinates of sampling locations and the essential oil percentage of *Achillea aucheri* growing at different altitudes in Mount Damavand.

Altitudes (Meter)	Latitude	Longitude	July daily average temp (ºC)	Essential oils (% w/w DW)
3900	35° 55' 32.3" N	52° 6' 26.8" E	26	0.681 ± 0.04
4000	35° 55' 36.5" N	52° 6' 29.6" E	25.5	0.762 ± 0.03
4100	35° 55' 41.3" N	52° 6' 34.0" E	24	0.856 ± 0.02
4200	35° 55' 47.5" N	52° 6' 33.8" E	22.5	0.949 ± 0.04
4300	35° 55' 54.0" N	52° 6' 32.3" E	20	0.954 ± 0.07

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Table 2. Essential oil compositions and their percentage in *Achillea aucheri* growing at different altitudes in Mount Damavand.

No.	Compounds	*RI*[a]	3900 m	4000 m	Altitudes 4100 m	4200 m	4300 m
1	Tricyclene	927	0.10	0.11	0.20	0.26	0.35
2	α-Thujone	930	2.92	2.20	1.27	1.09	0.91
3	α-Pinene	939	2.20	2.45	3.40	3.50	4.20
4	Camphene	954	4.43	4.17	3.39	2.55	1.48
5	Sabinene	975	0.54	0.75	0.83	0.89	0.99
6	α -Fenchene	982	4.69	4.33	3.86	3.00	2.11
7	Myrcene	990	0.10	0.20	0.70	1.10	1.70
8	Delta-2-carene	995	0.56	0.50	0.43	0.33	0.20
9	Yomogi alcohol	998	9.50	10.20	11.50	11.60	11.80
10	M-Cymene	1026	3.20	2.70	2.70	2.20	2.10
11	P-Cymene	1027	1.23	1.08	1.08	0.73	0.74
12	Limonene	1030	1.26	1.65	1.90	2.20	3.65
13	1,8-Cineole	1031	7.34	5.83	5.60	3.85	2.29
14	(Z)- ß-Ocimene	1140	11.47	10.56	9.37	9.14	8.64
15	(E)- ß-Ocimene	1051	1.28	0.70	0.60	0.52	0.11
16	γ-Terpinene	1060	0.19	0.21	0.20	0.20	0.67
17	Linalool	1084	6.78	8.05	3.20	1.20	-
18	γ-Terpinolene	1089	1.93	2.45	3.38	4.62	5.92
19	Perillene	1102	0.14	0.14	0.18	0.22	0.33
20	Isopropyl thiobenzen	1126	1.20	0.87	0.62	-	-
21	Chrysanthenone	1128	2.92	3.10	4.36	8.35	9.28
22	Camphor	1145	3.20	4.40	6.20	6.96	7.47
23	Isopulegol	1150	15.84	16.05	16.57	16.67	16.80
24	cis-Chrysanthenol	1164	-	0.10	0.70	0.90	1.10
25	Pinocarvone	1168	0.24	0.33	0.32	0.36	-
26	α-Terpineol	1189	4.85	4.50	2.70	0.98	0.50
27	cis- Trans anethol	1203	0.23	0.21	0.20	0.20	0.14
28	Fragranol	1210	1.64	0.90	0.43	0.33	0.23
29	3-Nonanone	1214	0.45	0.41	0.38	0.28	0.24
30	α -Carvone	1248	0.55	0.44	0.44	0.41	0.40
31	Cumin aldehyde	1251	0.54	0.42	0.40	0.12	0.10
32	Thymol	1288	0.10	0.10	0.40	0.26	0.29
33	Carvacrol	1299	0.23	0.24	0.19	0.13	0.11
34	Methyl eugenol	1398	0.95	0.50	0.31	0.34	0.33
35	3-Chloroacetylacetone	1438	0.35	0.24	0.24	0.22	0.16
36	Nerolidol	1588	0.87	1.16	2.42	4.16	4.10
37	α-Eudesmol	1654	0.30	0.27	0.60	0.50	0.20
38	Chamazulene	1732	0.30	0.70	1.70	2.50	3.50
39	α -Oxobisabolone	1748	0.41	0.57	0.34	0.28	0.10
40	n-Octadecan	1802	0.26	0.32	0.55	0.54	0.60
41	Methyl palmitoleate	1859	1.20	1.29	1.16	1.11	1.07
42	Hexadecanol	1875	-	0.13	0.20	0.21	0.27
43	Phytol	1942	-	0.55	0.49	0.34	0.33
44	Eicosane	1998	0.20	0.17	0.19	0.20	0.20
45	Octadecanol	2081	-	-	0.20	0.27	0.44
	Monoterpene hydrocarbons		34.3	31.2	29.43	26.82	23.2
	Oxygenated monoterpenes		42.39	44.3	43.7	43.71	45.86
	Sesquiterpene hydrocarbons		2.98	2.92	4.27	5.44	6.69
	Oxygenated sesquiterpenes		15.2	16.22	17.11	18.25	18.86
	Diterpens		1.82	1.62	1.61	1.56	1.49
	Total		96.69	96.26	96.12	95.78	96.1

[a] Kovats Retention Index (RI).

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components were identified, on average. In general, the main components of the oil were isopulegol (16.39%), yomogi alcohol (10.92%), (Z)-*ß*-ocimene (9.84%), camphor (5.65%), 1,8-cineole (4.98%), linalool (4.81%), *γ*-terpinolene (3.66%), *α*-fenchene (3.60%), camphene (3.20%), *α*-pinene (3.15%), *α*-terpineol (2.71%), nerolidol (2.54%), chamazulene (1.74%) and *α*-thujone (1.67%). The results also showed a high content of monoterpene compounds (72.98%) and low percentage of sesquiterpenes (21.58%) in oils. The oil of *A. aucheri* was characterized by large content of oxygenated monoterpenes (43.99%) with isopulegol and yomougi alcohol being the major constituents. The monoterpene hydrocarbons (28.99%) with (Z)-*ß*-ocimene and α-fenchene being the most important ones.

We identified and quantified about 45 components of *A. aucheri* essential oil. These components are many more than those reported by Rustaiyan *et al*. (1999), who identified 22 different oil compounds in *Achillea aucheri* Boiss. In general, yomogi alcohol, (Z)-ß-ocimene, camphor and 1,8-cineole were the major chemical compounds of *A. aucheri*. Supporting this, Dokhani *et al.* (2005) reported 1,8-cineole as one of the major compounds of the *A. millefolium, A. eriophora* and *A. tenuifolia* essential oils.

From the chemical point of view, the essential oil composition frequently changed as a result of altitudes variation. There were some components such as isopulegol, yomogi alcohol, chrysanthenone, *γ*-terpinolene, nerolidol, *α*-pinene and chamazulene which showed the highest and lowest percentage in the altitude of 4,300 and 3,900 m, respectively. In agreement with that, Haider *et al.* (2010) showed that the main constituents of the oil from *Artemisia nilagirica* had variation with changes in altitude.

We demonstrated the high percentage of *A. aucheri* oil at lower altitudes. However, α-thujone, a harmful compound of the oils, at lower altitude was three times more concentrated in comparison with higher altitudes. Supporting this, Giorgi *et al.* (2005) showed that the main constituents of the oil from *Achillea millefolium* L. had variation with changes in altitude and *α*- and *β*-thujone were not present in oils from fields at a higher altitude. Haider *et al.* (2009) revealed that the oils from *Artemisia roxburghiana* collected from the lower altitudes yielded higher percentage of oils (0.8–0.85%) which were dominated by α-thujone. It is noticeable that the median Lethal Dose (LD_{50}) of *α*-thujone, the more active within its two isomerises, is nearly 45 mg kg^{-1} in mice and with 100% mortality rate at 60 mg kg^{-1}. From 30 to 45 mg kg^{-1}, the mice experience muscle spasms in the legs, which progress to general convulsions until death or recovery (Höld *et al.,* 2000).

Noticeably, some of the components of *A. aucheri* essential oil reported by Rustaiyan *et al*. (1999) were not seen in our chromatogram analysis. This variation might be related in part to geographical differences among the sampling locations. In general, genetic differences are much higher than those caused by varying environmental conditions. Polymorphism is often found when the essential oil composition of individual plants from a given species is compared with each other (intraspecific variation or 'chemotype'). This polymorphism is based on the genetic background of the species (Odeta and Petras, 2007; Azizi *et al.,* 2010). Taken all together, it seems that the studied accession of *A. aucheri* in current research is most likely a new chemotype of this plant species.

The results presented here are clarifying the difference in the composition of essential oil of *A. aucheri* as a result of the plant response to environmental conditions at different altitudes. Altitude, as an important factor, affects ecosystem conditions such as temperature, relative humidity, water availability, and sunlight intensity, which all together have definite impact on oil compositions and their quantity (Rahimmalek *et al.,* 2009; Abad *et al.,* 2012). The major components at altitude of 3900 m were isopulegol and (Z)-*ß*-ocimene,

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at the expense of a considerable decrease in (Z)-*β*-ocimene at altitude of 4,300 m, at which the major components were isopulegol and yomogi alcohol. The results showed that chamazulene content, the most important component of oil, increased exponentially from 3,900 to 4,300 m altitude, more than 10 times. This could influence its specific medicinal effect which is related to presence of chamazulene.

CONCLUSIONS

We identified and quantified about 45 components of *A. aucheri* essential oil which were much more than those reported previously. There were some differences between the composition of the analyzed oil in this study and previously reported one for the same species. Hence, an accession of *A. aucheri* endemic to Mount Damavand could be considered as a new chemotype in this species. The results showed that the essential oil composition of *A. aucheri* varied at different altitudes, as the result of climatic factors variation. From this point of view, some new compounds could be introduced or their value could be changed in useful or harmful way·

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بررسی ترکیبات اسانس بومادران دماوندی (*Achillea aucheri* Boiss) در ارتفاعات رشدی مختلف دماوند، ایران

ا. فرهنگ سردرودی، ع.سلیمانی، ع. خیری، و ر. زیباسرشت

چکیده

جنس بومادران (*Achillea*) یکی از مهمترین جنس‌های گیاهان دارویی در خانواده گیاهی کاسنی (Asteraceae) می‌باشد. تعداد ۱۹ گونه گیاهی از این جنس در فلور ایرانی وجود دارد که در این بین بومادران دماوندی بومی ایران می‌باشد. با توجه به تاثیر مکان جغرافیایی رشد بر کمیت و کیفیت اسانس، تحقیق حاضر به منظور تعیین اثر ارتفاعات رشدی مختلف در منطقه کوه دماوند بر محتوا و اجزاء اسانس این گیاه صورت گرفت. استخراج اسانس با استفاده از روش تقطیر با آب صورت گرفت و بطور میانگین، حدود ۹۶/۲۰ درصد از ترکیبات آن با استفاده از دستگاه GC و GC/MS شناسایی شدند. ترکیباتی از قبیل ایزوپولگول (۱۶/۳۹ ٪)، یوموگی الکل (۱۰/۹۲ ٪)، (Z)- بتا- اوسیمن (۹/۸۴ ٪)، کامفور (۵/۶۵ ٪)، ۱، ۸- سینئول (۴/۹۸ ٪)، لینالول (۴/۸۱ ٪)، گاما ترپینولن (۳/۶۶ ٪)، آلفا فنچن (۳/۶۰ ٪)، کامفن (۳/۲۰ ٪)، آلفا پینن (۳/۱۵ ٪)، آلفا ترپینئول (۲/۷۱ ٪)، نرولیدول (۲/۵۴ ٪)، کامازولن (۱/۷۴ ٪) و آلفا توجون (۱/۶۷ ٪) به همراه ۳۴ ترکیب دیگر شناسایی گردیدند. در کل شرایط متغییر آب و

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هوایی ناشی از ارتفاعات مختلف، بر ترکیب اسانس گیاه بومادران دماوندی تاثیر داشت. با افزایش ارتفاع درصد ترکیب آلفا توجون، از ترکیبات مضر برای سلامتی انسان، در اسانس کاهش نشان داد. بعلاوه ترکیب ارزشمند کامازولن با افزایش ارتفاع از ۳۹۰۰ به ۴۳۰۰ متر، بیش از ۱۰ برابر افزایش نشان داد.

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