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Original Article

Eessential Oil Composition of *Achillea filipendulina, A. arabica* and *A. eriophora* Cultivated under Temperate Climate in Iran

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Abstract

The *Achillea* L. genus has a wide distributional range, and the differences in oil composition may be affected by different environmental factors such as soil mineral fertilization, the climate conditions and the culture site. To evaluate the effect of temperate climate on content and chemical combination of essential oil of *Achillea filipendulina* Lam, *A. arabica* Kotschy and *A. eriophora* DC., this study was carried out. Essential oils were analyzed by GC and GC/MS. Yield of essential oil of *A. filipendulina*, *A. arabica* and *A. eriophora* cultivated was 0.9, 2.25 and 2.3%, respectively .The major components of *A. filipendulina* were santolina alcohol (37.2%), borneol (12.7%),1,8- cineole (8.7%), germacrene D (6.22%), and camphor (4.09%). In *A. arabica*, the major compounds were *p*-cymene (14.61%), piperitone (13.09%), camphor (12.81%) and 1, 8-cineole (11.97%). In *A. eriophora*, principal compounds were camphor (19.57%), 1, 8-cineole (19.06%), camphene (9.61%) and α-pinene (7.35%). Our results demonstrate variations in the qualitative and quantitative composition in the oils obtained from the aerial parts of the studied species. Also, chemical profile of *A. filipendulina* was slightly affected under temperate climatic conditions, suggesting its ability and flexibility to be cultivated in temperate zones.

Keywords: *Achillea*, Essential oil composition, Temperate climate

Introduction

The genus *Achillea* L. belongs to the family Asteraceae and comprises of about 115 species. *Achillea* L. genus is native to Europe, Western Asia and North Africa, although they are also found in Australia, New Zealand and North America. It has been represented in Iran by nineteen species including seven endemics [1]. One of Iranian endemic species is *A. eriophora*, occurring mainly in the south, at altitudes between 700 and 3000 m. The essential oil of this species showed some *in vitro* antimicrobial activity. *A. arabica* occurs naturally in many parts of Iran in the central, north, northwest, west and northeast with the local name of 'Bumadarane Zard'. This plant is a perennial villous herb with 10 – 100 cm height and radiate heads which are borne in large dense compound corymbs on the erect stems [1]. To date, many investigations considered the volatile oil of *A. arabica* from the chemical constituents to biological activities points of view [2]. Based on the results of these studies, there is a considerable chemical polymorphism in the essential oil of this plant. These oils show different biological activities including antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing [3]. *A. filipendulina* is distributed in Central Asia, the Caucasus and Iran. This plant, locally named

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"Buimodaron", grows 80-120 cm and flowers from June to August, and has been used since ancient times in traditional herbal medicines for a variety of ailments. Decoctions of *A. filipendulina* have been used to treat sciatica, gout, arthritis, gastrointestinal disturbances, congestion, cardiovascular diseases, and malaria, as well as a diuretic, anthelmintic and purgative. Externally, the plant has been used to treat scabies and wounds [2].

As Hassandokht *et al.* [4] reported, on the dry weight of samples, the essential oil percentage of *A. arabica* ranged from 0.41% (whole plant in fruit set stage) to 1.12% (flowers in full flowering stage). In other studies, the essential oil content of *A. arabica* collected from different parts of the world at the flowering stage has been reported to be 0.20% in Jordan [5], 0.63–0.70% in Turkey [3], 1% in Iran [6] and 0.42–2.70% in Iran [7]. Azizi *et al.* [8] reported the yield of essential oil of *A.eriophora* about 2.2%. Ghani *et al.* [9] showed that essential oil yield of *A. eriophora* when grown in wild and cultivated conditions were 2% and 2.25% (v/w) respectively. Mosayebi *et al.* [10] reported content of essential oil of *A. filipendulina* from 3 areas of Iran with average 0.5 to 0.7%.

Tabanca *et al.* [11] reported the main components of essential oil of *A. arabica* from Turkey as piperitone (31.06%), eucalyptol (10.98%) and camphor (12.46%). Dehghan, *et al.* [12] showed the major compounds of *A. arabica* were α-terpinen (41.42%), delta-2-carene (13.96%), *p*-cymene (13.41%) and 1,8-cineole (8.91%) in East-Azarbayjan province, Iran. In another study, Morteza-semnani *et al.* [13] reported that the main constituents of the oil of *A. arabica* collected from Mazandaran province, Iran, were 1,8-cineole (7.9%), camphor (6.5%), α-fenchene (5.7%) and santolian triene (5.1%). Azizi *et al.* [8] reported 1,8-cineol as main compound in *A. arabica*, ranging from 26-36% in the flower heads and *p*-cymene and camphor, piperitone, ascaridol and isoascaridol were further major components of this species. Maghsoodlou *et al.* [14] pointed out Camphor (21.55 %), Artemesia ketone (13.84%), Alpha-Thujone (11.85%), Borneole (8.94%), Yomogi alcohol (7.74%), 1,8-cineole (5.19%), Terpinene-4-ol (4.23%) and Myrtenol (3.10%) as the major constituents of essential oil compounds in Iranian *A. eriophora*. Also, Azizi *et al.* [8] reported camphor (33-36%) and 1,8-cineol (25-30%) as main compounds and α-pinene, sabinene, camphene, β-pinene, borneol, α-terpineol and terpinen-4-ol in lower amounts in Iranian populations *of A. eriophora*. Mosayebi *et al* [10] reported that samples of *A. filipendulina* from Iran were dominated by santolina alcohol (43.6-47.8%) with lesser amounts of 1,8-cineole (4.1-8.1%) and borneol (3.9-9.1%). Rahimmalek and Zeinali [15] have reported essential oils of Iranian populations of *A. filipendulina* contain santolina alcohol (23.4-24.1%), borneol (7.9-8.3%), bornyl acetate (11.6-14.7%) and germacrene D (11.8-23.4%). In Tajikistan, the major components of *A. filipendulina* oil were reported as santolina alcohol (43.6-46.3%), 1,8-cineole (8.8-11.4%), borneol (5.3-6.0%), isoborneol (4.8-5.4%), and cischrysanthenyl acetate (6.5-9.3%) by Sharopov and Setzer [16]. Also, as Adekenov [17] reported essential oil of *A. filipendulina* from Kazakhstan was composed largely of santolina alcohol (29.0%), borneol (27.8%), 1,8-cineole (19.1%), and bornyl acetate (8.1%). santolina alcohol (43.6-46.3%), 1,8-cineole (8.8-11.4%), borneol (5.3-6.0%), isoborneol (4.8-5.4%), and cischrysanthenyl acetate (6.5-9.3%).

As reported, the biosynthesis of secondary metabolites including the volatiles in medicinal and aromatic plants is influenced by various agricultural practices and environmental factors namely the soil mineral fertilization, the climate conditions and the culture site [18].

According to a report published by Botanical Gardens Conservation International (BGCI) in 2008, about 15,000 species of medicinal plants are now threatened by extinction caused by a lot of factors, specially *over-harvest* of rangelands. As above-mentioned organization recommended attempt to culture these plants in farms to commercial purposes *has been seen necessary* [19]. The current paper presents the content and the results of GC-MS analyses of the essential oils from aerial parts of *A. filipendulina*, *A. arabica* and *A. eriophora* in cultivated condition in Karaj, as a sample of a temperate climate.

Material and Methods

Plant Material and Field Experiments:

The plants of *A. arabica* were collected from "Shahrood" in Semnan province, *A. eriophora* from "Khatam" in Yazd province, and *A. filipendulina* from "Khalkhal" in Ardabil province, Iran in 2009 and their seeds had been reserved in National Natural Resources Gene Bank, Iran.

The seeds were cultivated in pots in the greenhouse at 23-26°C for seedling production. When the seedlings had developed 6 true leaves, were transplanted to the research field in Karaj, Iran. The plots (2×2 m with 35 and 50 cm within and between the rows, respectively)

were arranged in a Randomized Complete Block Design (RCBD) with three replications.

The soil of the experimental field was loamy with pH= 7.5-8.5. The organic carbon content of the soil was 0.25% (w/w). The soil had 125 kg/ha available nitrogen, 25 kg/ha available phosphorus and 122kg/ha available potassium. The cultural operations consisted of manual elimination of weeds, frequent irrigation in order to maintain the soil wet and fertilizer administration.

Extraction of Essential Oil

For the extraction of essential oil of aerial parts, the plants were harvested at full bloom stage. Dried plant material (40g) was subjected to hydro-distillation for 3h using a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature before analysis.

GC and GC–MS Conditions

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30m × 0.25 mm id., film thickness 0.25 µm). Oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate 3°C/min. Injector and detector (FID) temperatures were 290°C; helium was used as the carrier gas with a linear velocity of 32 cm/s. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d, film thickness 0.25 µm.). Oven temperature was 40-240°C at a rate of 3°C min, injector temperature 250°C and transfer line temperature 260^{o}C , carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV, scan time 1s, mass range 40-300 amu.

Identification of Volatile Components

The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices (RI), either with those of authentic compounds or with data published in the literature [20].

Results

Hydro distillation of the aerial parts of *A. filipendulina*, *A. arabica* and *A. eriophora* yielded 0.9, 2.25 and 2.3% (w/w) of essential oils, respectively.

In table 1 the compounds identified are listed according to their retention, and are classified in five classes on the basis of their chemical structures. As Table shows the composition of the essential oils is different among *Achillea* species, both qualitatively and quantitatively. While the highest percent of monoterpene hydrocarbons is belonged to *A. arabica*, *A. eriophora* and *A. filipendulina*, respectively, the highest percent of oxygenated monoterpenes is belonged to *A. filipendulina*, *A. eriophora* and *A. arabica*, respectively. The oil obtained from all of species, is characterized by a low concentration of sesquiterpenes that represents less than 15% of the composition of the oil in *A. arabica*, and a significantly lower amount in other two species.

Overall, 28 components were identified of total essential oil of *A. filipendulina* L. The major components of *A. filipendulina* were santolina alcohol (37.2%), borneol (12.7%), 1,8- cineole (8.7%), germacrene D (6.22%), and camphor (4.09%). A total of 26 components of the essential oil of *A. arabica* aerial parts were identified (Table 1). The principal compounds in *A. arabica* oil were *p* -cymene (14.61%), piperitone (13.09%), camphor (12.81%) and1, 8-cineole (11.97%). In the essential oils of *A. eriophora* 24 components were identified, (Table 1), that principal compounds were camphor (19.57%), 1, 8-cineole (19.06%), camphene (9.61%) and α-pinene (7.35%).

Discussion

In our study, yield of essential oils for all of species showed relative superiority in compare with previous researches that were done in grown conditions. Higher amount of content of essential oils in cultivated statute than grown condition was reported by Ghani *et al.* [9] in *A. eriophora.* Fertilizers and irrigation have been found to increase the yield of essential oil from established crops like *Artemisia annua*, *Chamomilla recutita*, *Rosmarinus officinalis*, *Valeriana officinalis* [21].So, it could be said that suitable nutrient status of soil and regulated irrigation increased content of essential oils of *Achillea* species in our study.

According to the literatures, monoterpenes are the principle components of *Achillea* essential oils. Also, 1, 8-cineole, camphor, borneol, α- and β- pinenes are among the five most abundant monoterpene components [22]. In recent research monoterpenes are about 86, 80 and 84.6% in *A. filipendulina*, *A. arabica* and *A. eriophora*. About most abundant monoterpene components, although 1, 8- cineole, camphor and borneol were among the five most a

monoterpene components in all of species, α- and β- pinene just were among high ranks in *A. eriophora* and *A. filipendulina*, respectively .

Table 1 Composition (%) of essential oils from aerial parts of *A. filipendulina*, *A. arabica* and *A. eriophora*

Monoterpene Hydrocarbons				
RI	Component	*A. filipendulina*	*A. arabica*	*A. eriophora*
919	santolina triene	0.10	-	0.20
929	α-Thujene	-	0.45	-
935	α-pinene	0.20	0.51	7.35
948	Camphene	-	1.66	9.61
960	Sabinene	2.19	0.34	0.56
979	β-pinene	3.92	0.49	0.50
1026	*p*-cymene	1.85	14.61	0.40
1030	Limonene	2.81	-	-
1065	α-Terpinen	2.42	5.42	1.36
1089	Terpinolene	0.10	0.10	0.10
1328	Delta-2-carene	-	0.50	-
	Total (%)	13.59	24.08	20.08

Oxygenated monoterpenes				
RI	Component	*A. filipendulina*	*A. arabica*	*A. eriophora*
916	artemisia alcohol	0.20	-	-
1033	1,8- cineole	8.60	11.97	19.06
1057	Santolina alcohol	37.2	-	-
1062	Artemisia ketone	-	0.10	-
1067	*Trans*-sabinene hydrate	-	1.80	-
1069	cis-sabinene hydrate	0.74	0.34	-
1098	Linalool	-	6.10	1.60
1124	α-campholenal	1.10	0.36	-
1141	Camphor	4.09	12.81	19.57
1153	Menthone	0.10	-	-
1160	Pinocarvone	1.43	-	0.87
1165	Borneol	12.7	6.53	6.71
1171	*Cis*-pinocamphone	-	0.71	-
1176	Terpinen-4-ol	0.65	-	3.38
1189	α-terpineol	1.88	0.93	3.38
1191	Myrtenol	1.56	-	3.48
1237	Cis-sabinyl acetate	0.67	1.1	3.09
1250	Piperitone	1.43	13.09	-
1330	*Trans*- carvyl acetate	-	-	3.36
	Total (%)	72.35	55.84	64.5

Sesquiterpene Hydrocarbons				
RI	Component	*A. filipendulina*	*A. arabica*	*A. eriophora*
1418	E- caryophyllene	-	-	1.04
1500	Bicyclogermacrene	1.32	4.51	2.81
1718	Germacrene D	6.22	9.73	1.61
	Total (%)	7.54	14.24	5.46

Oxygenated Sesquiterpenes				
RI	Component	*A. filipendulina*	*A. arabica*	*A. eriophora*
1577	Spathulenol	-	0.30	0.40
1582	Caryophyllene oxide	-	-	0.75
1624	α-eudesmol	0.43	0.72	-
	Total (%)	0.43	1.02	1.15

Other				
RI	Component	*A. filipendulina*	*A. arabica*	*A. eriophora*
1120	*Cis*-ment-2-en-1 ol	1.27	-	-
1283	Bornyl acetate	0.35	-	5.37
1354	Eugenol	1.20	4.24	-
	Total (%)	2.82	4.24	5.37
	Total identified (%)	96.73	99.42	96.56
	Total unidentified (%)	3.27	0.58	3.44

In contrast to other researches, santolina alcohol in *A. filipendulina*, and *p*-cymene, as well, piperitone in *A. arabica* were in the highest ranks; also, camphene was among the most abundant monoterpene components in *A. eriophora*. *Achillea arabica:* The essential oil of *A. arabica* displayed, *p* -cymene, piperitone, camphor and 1,8-cineole as its main compounds. According to available literature reports, the essential oil of *A. arabica* may vary greatly with the respective origin. For example, in turkey, piperitone, eucalyptol and camphor were reported as main compounds [11]. In Iran, α-terpinene, Delta-2-carene, *p* -cymene and 1, 8-cineole [12], 1, 8-cineole, camphor, α-fenchene and santolian triene [13], 1,8-cineol, *p*-cymene and camphor, piperitone, ascaridol and isoascaridol reported as major components of this species [11].

As above shows Iranian populations of *A. arabica* just have one common constituent in considerable amount, 1,8-cineole. We found some components which have not been found in Iranian *A. arabica* previously, for example, Delta-2-carene, artemisia ketone and eugenol, although they were in inconsiderable amount. These differences might be due to the diversity of the plant sources, different essential oil hydrodistillation procedures or cultivation conditions.

Achillea eriophora: The volatile fraction of *A. eriophora*, contained two main compounds: camphor,1, 8-cineole. Other main constituents in fewer amounts were camphene and α-pinene. In Iranian *A. eriophora*, camphor, 1,8- cineol, α-pinene, sabinene, camphene, β-pinene, borneol, α-terpineol and terpinen-4-ol [8] and camphor, artemesia ketone, alpha-thujone, borneole, yomogi alcohol, 1,8-cineole, terpinene-4-ol and myrtenol [14] were reported as the major constituents.

According to above- mention results it could be concluded that *A. eriophora* populations have at least two common constituent in considerable amount, camphor and 1,8-cineole. Although santolina alcohol, yomogi alcohol and *cis*-chrysanthenol were found in other studies, they could not be detected in the plants from the present study. On the other hands, some components were found for first time in our study, for example, santolina triene and spathulenol.

A. filipendulina: According to our data santolina alcohol, borneol, 1,8-cineole, germacrene D, and camphor were the main constituents of *A. filipendulina*. Our results are qualitatively consistent with the other reports about the main constituents of *A. filipendulina* [10, 13]. The high concentrations of santolina alcohol, 1,8-cineole, and borneol in *A. filipendulina* as revealed in this study and previous works likely account for the traditional uses of this plant for treatment of infections, inflammation, etc. Santolina alcohol, 1,8-cineole, and borneol have shown antimicrobial effects. Additionally, 1,8-cineole and borneol have shown synergistic effects, likely due to penetration enhancement [2].

Although isoborneol and cischrysanthenyl acetate were reported in some studies as main compounds of *A. filipendulina* [16], they could not be detected in present study. On the other hands, eugenol was only discovered compound in our study which had been not reported in other researches. Eugenol is commonly used by dentists because it is antiseptic and anti-inflammatory. Also it was showed that eugenol is a very powerful fat-soluble antioxidant, inhibiting the accumulation of fat peroxide products in red blood cells and maintaining the activities of the body's antioxidant enzymes at normal levels [6].

Comparison of the three species: According to the composition of the essential oils, the greatest similarities were between *A. arabica* and *A. eriophora*, where among the 15 most abundant components, they showed ten common components (camphor, 1,8- cineole, camphene, borneol, α-terpineol, cis- sabinyl acetate, bicyclogermacrene, germacrene D, linalool and α-terpinen) that totally were about 60.8 and 69% in *A. arabica* and *A. eriophora*, respectively. Santolina alcohol which was predominant in *A. nobilis*, could not be found in the other species. *P*-cymene, the first major compound in the oil from *A. eriophora*, was present as a minor compound in the other two species. piperitone the second major compound in the oil from *A. eriophora*, although was found as a minor compound in the *A. filipendulina*, could not be found in *A. arabica*.

Conclusion

In conclusion, our results demonstrate variations in the qualitative and quantitative composition in the oils obtained from the aerial parts of *A. filipendulina, A. arabica and A. eriophora* cultivated in one zone. These variations may be due to the influence of geographical differences, physiological differences and genetic factors [9]. Comparing results with findings from other studies about mentioned *Achillea* species in wild (grown) conditions showed that oil content increased in cultivation situation. Also, chemical profile of *A. filipendulina* was slightly affected under temperate climatic conditions of Karaj,

suggesting its ability and flexibility to be cultivated in temperate zones.

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