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Chemical Compositions of Essential Oils of Different Parts and Extract of *Achillea santolina* L. from Iran

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GC and GC-MS analysis of the isolated essential oils obtained by steam distillation from the flowers, leaves and stem of *Achillea santolina* L., known to have medicinal activity, collected from Iran. The constituents obtained by hexane-ether extraction of the aerial parts of *Achillea santolina* L. resulted in the identification of 48-59 constituents (96.1-98.0 % of the total oils and extract). Different parts of *A. santolina* were the valuable organs for the essential oil production representing a yield of 0.15-0.7 % (w/w). In the hydrodistilled oil of three parts and the extract of *A. santolina*, 1,8-cineole (3-5 %), camphor (3.8-5.1 %), terpinene-4-ol (4.0-7.1 %), fragranol (7.1-9.1 %), fragranyl acetate (20-37 %), α-terpinyl acetate (0.6-5.1 %), caryophyllene oxide (1.2-3.2 %), α-muurolol (1.1-3.6 %) and some alkanes, alkanoic acids and esters were principle components, also monoterpenes (7.1-9.1 %) and oxygenated monoterpenes (55.1-77.2 %) were dominant components.

Key Words: *Achillea santolina* L., Essential oil composition, Solvent extract, Fragranyl acetate.

INTRODUCTION

The genus *Achillea* L. (Asteraceae) is represented by about 115 species found in the northern hemisphere, mostly in Europe and Asia and commonly known as yarrows[1-3]. It has been represented in Iran by nineteen species including seven endemics, among this genus, *Achillea santolina* L. (it has been known as Bomadaran in Iran) is represented in Iran[4]. The *Achillea* L. species belong to the oldest medicinal plants that are used both for pharmaceutical purposes and in folk medicine.

These plants contain a complex of different pharmacological compounds, for example, terpenes, flavonoids, alkaloids, bitters, tannins, lignans[5].

Achillea species are diuretic, emmenagog agents, wound healing, for curing stomachache, diarrhea and antichloristic, antispasmodic, antiseptic and infection preventing properties and have been used to reduce sweating and to stop bleeding, amarum, stomachicum, cholagolum and carminativum, too[3,6-13]. The *Achillea* genus has a wide distributional range[14] and the differences in oil composition may be affected by different environmental factors, plant genetic type, seasonality and developmental stage, because of a chemically polymorphic and perennial[15].

Terpenoids (1,8-cineole, camphor, borneol, pinenes, artemisia ketone, santolina alcohol, farnesane, caryophyllene and its oxides, cubebene, germacrenes, eudesmol, α-bisabolol and oxides, farnesene, γ-gurjunene, γ-muurolene and chamazulene) are the principle components of *Achillea* essential oils[3,13].

Chemical composition of the essential oils of five *Achillea* species from Turkey[6], also essential oils and hexane extracts of *A. frarantissima* and *A. santolina* from Egypt[16], composition at different development stages of the essential oil of four *Achillea* species grown in Iran[17] and composition of the essential oil of *A. wilhelmsii* collected from Kazeroon in Fars province, Iran[18] were established by GC/MS and GC. There are few reports about essential oil composition of *A. santolina* compared with another *Achillea* genus[19,20]. In the present study, the flowers, leaves and stems of the *Achillea santolina* L. collected from Khorasan Razavi province, Iran, were investigated for their essential oil compositions as well as hexane-ether extract, which analyzed by both gas chromatography and gas chromatography mass spectrometry.

EXPERIMENTAL

Achillea santolina L. was collected from Jannatabad (latitude +35°35'56.30"N, longitude +61°8'25.97"E) in Torbat Jam, Khorasan-Razavi Province, Iran, in June 2011. The plant was air dried and dried sample was crushed, then essential oils were obtained by hydrodistillation (HD) of their flowers,

leaves and stem, separately. Aerial part of plant for extract was dried and crushed. Voucher specimens of the plant have been deposited in the Ferdowsi University of Mashhad herbarium (FUMH), Mashhad, Iran, by Prof. Javad Ghoreishalhosseini.

Sample preparation: The flowers, leaves and stems of *A. santolina* L. were separately subjected to hydrodistillation for 4 h using an original Clevenger-type apparatus and yielded essential oils from 0.15 to 0.7 % (w/w) of dry matters. After decanting, the obtained essential oils were dried over anhydrous sodium sulfate and after filtration, stored in refrige-rator at -4 ºC until tested and analyzed.

For extract preparation, the aerial parts of plant (*ca.* 50 g) were shaken sequentially in percolation with hexane-ether (1:1, v/v) for 16 h (15 mL/g) at room temperature. Sample was sonicated for 15 min twice and then solvents were removed subsequently under reduced pressure by rotary evaporator apparatus. The extract was weighed and stored in refrigerator at 4 ºC until tested; the yield was 1.2 % (Table-1).

TABLE-1
WEIGHT OF DIFFERENT PARTS OF PLANT, TIME AND YIELD (%) OF HYDRODISTILLATION (HD)

Parameter	Weight (g) out of dry matter	Time (h)	Yield (%)
Flower oil	110	4	0.70
Leaf oil	125	4	0.25
Stem oil	107	4	0.15
Hexane-ether extract	50	16	1.20

Gas chromatography: The essential oils were diluted (1/100 in hexane, v/v) and 1 µL were injected manually and in the splitless mode were used for analysis. GC-MS analyses of the essential oils and extracts was analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091) 413:325 ºC, HP-5 column (30 m × 320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 mL/min. The GC oven temperature was initially programmed at 50 ºC (hold for 1 min) and finally at 300 ºC (hold for 5 min) at a rate of 8 ºC/min while the trial temperature was 37.25 ºC.

The column heater was set at 250 ºC in a splitless mode while the pressure was 10.2 psi with an average velocity of 66.5 cm/s and a hold-up time of 0.75 min. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250 ºC.

Gas chromatography-mass spectrometry: The essential oils and extract were analyzed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091) 413:325 ºC, HP-5 column (30 m × 320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 mL/min. GC oven temperature and conditions were as described above. The injector temperature was at 250 ºC. Mass spectra were recorded at 70 eV. Mass range was from m/z 30 to 500. Diluted samples (1/100 in hexane, v/v) of 1 µL were injected manually and in the splitless mode.

Detection method: The identification of the components were based on their retention indices data determined by reference to a homologous series of *n*-alkenes (C_9-C_{28}) and by comparison of their mass spectral fragmentation patterns with those of authentic compounds or with data published in the literature as described by Adams[21]. Further identification was made by matching their recorded mass spectra with those stored on the MS library (NIST 08.L database/chemstation data system). Determination of the percentage composition was based on peak area normalization without using correction factors.

RESULTS AND DISCUSSION

Essential oils and extract composition: The essential oil were obtained from the crushed flowers, leaves and stems by separately hydrodistillation. The hexane-ether extract was obtained from aerial parts of *A. santolina*, which were immediately analyzed by both gas chromatography and gas chromatography-mass spectrometry system. The results are presented in Table-2.

A total of 53, 48 and 50 chemical constituents, representing 96.1, 98.0 and 96.3 % of the total content, were identified in each investigated essential oils isolated from flower, leaf and stem, respectively. 50 g out of aerial parts extracted by a mixture (1:1) of hexane-ether followed by GC and GC-MS analysis, 59 (97.2 %) components were characterized. In addition, the essential oils were also found to be rich in oxygenated monoterpenes (> 72 %), while solvent extract showed minor amount (55.1 %). In essential oils monoterpenes (8.3-9.1 %), sesquiterpenes (3.0-4.7 %) and oxygenated sesquiterpenes (5.2-7.4 %) were less. Hexane-ether extract of *A. santolina* showed 7.1 % monoterpene hydrocarbon, 4.5 % sesquiterpene hydrocarbon, 14.1 % oxygenated sesquiterpene and 16.5 % non-terpene compounds. 1,8-cineole (3.0-5.0 %), Z-sabinene hydrate (2.1-3.5 %), camphor (3.8-5.1 %), terpinen-4-ol (4.0-7.1 %), fragranol (7.1-9.1 %), fragranyl acetate (20.0-37.0 %), α-terpinyl acetate (0.6-5.1 %), caryophyllene oxide (1.2-3.2 %), α-muurolol (1.1-3.6 %) and some alkanes, alkanoic acids and esters were major components in oils and solvent extract. Hexadecanoic acid (5.2 %), eicosane (4.2 %) and methyl linoleate (3.1 %) only investigated in hexane-ether extract.

Essential oils of flower, leave and stem of *A. santolina* from Egyptian plant have been studied and 44 (97.62 %), 43 (95.62 %) and 37 (96.09 %) compounds identified respectively. Fragranyl acetate (45-52 %), fragranol (11-19 %), borneol (3-5 %), terpin-4-ol (6-7 %) and camphor (3-4 %) were the main constituents in the investigated parts[16]. The principle components in the hexane-ether extract of the aerial parts were fragranyl acetate (50.7 %), fragranol (8.78 %) and camphor (6.6 %). Mainly oxygenated monoterpenes were identified as the major constituents. Some constituents were observed in same amounts and were in agreement with our finding, but some constituents were identified by different percentages.

In *A. santolina* from Jordan, terpenoids (1,8-cineole, camphor, bomeol, pinenes, artemisia ketone, santolina alcohol, farnesane, caryophyllene and its oxides, α-bisabolol and oxides, cubebene, germacrenes, eudesmol, farnesene, γ-gurjunene, γ-muurolene and chamazulene) are the principle

TABLE-2
CHEMICAL COMPOSITION (%[a]) OF THE ESSENTIAL OILS OF FLOWERS, LEAVES AND STEMS, AND HEXANE-ETHER EXTRACT OF AERIAL PARTS ISOLATED FROM *A. santolina* IDENTIFIED BY KI[b] AND GC-MS[c]

No	Compound	KI[b]	Essential oil			Extract
			Flower	Leaf	Stem	
1	Santolina triene	908	0.1	0.2	–	0.2
2	α-Pinene	939	1.2	1.4	0.9	1.0
3	Camphene	954	1.4	1.3	1.2	0.8
4	Sabinene	957	–	–	–	0.9
5	β-Pinene	979	2.0	1.8	1.6	1.1
6	1,8-Dehydrocineole	991	Tr	0.1	–	0.1
7	α-Phellandrene	1002	0.1	–	0.2	0.4
8	α-Terpinene	1017	0.7	0.8	0.2	0.1
9	*p*-Cymene	1024	1.4	1.5	0.2	0.3
10	*o*-Cymene	1026	0.8	0.6	0.4	0.4
11	β-Phellandrene	1029	0.2	0.5	0.3	0.5
12	1,8-Cineole	1031	5.0	4.5	3.0	3.0
13	Santolina alcohol	1040	0.6	–	–	0.3
14	γ-Terpinene	1059	0.2	0.9	0.8	1.0
15	Artemisia ketone	1062	1.2	1.1	0.8	0.5
16	Z-Sabinene hydrate	1070	3.5	1.8	2.1	2.1
17	Artemisia alcohol	1083	2.1	0.6	0.5	1.1
18	Terpinolene	1088	0.2	0.1	0.1	-
19	Camphor	1146	4.2	4.1	3.8	5.1
20	Isoborneol	1160	0.9	0.8	0.6	0.5
21	Lavandulol	1164	0.6	–	0.3	0.1
22	Borneol	1169	1.5	3.5	4.5	0.6
23	Octanoic acid	1171	2.3	1.7	0.9	1.0
24	Terpinen-4-ol	1177	6.4	7.1	6.1	4.0
25	α-Terpineol	1188	0.5	0.5	0.4	0.5
26	Myrtenol	1195	0.5	–	0.2	0.7
27	Piperitol	1199	0.7	0.7	0.9	1.2
28	Fragranol	1215	8.1	9.1	7.8	7.1
29	Carvone	1243	Tr	–	–	-
30	Geraniol	1252	–	0.3	0.3	0.4
31	Z-Cinnamyl alcohol	1262	0.2	–	–	0.1
32	Geranial	1267	–	0.1	0.3	0.2
33	α-Terpinen-7-al	1282	0.9	–	–	-
34	Bornyl acetate	1288	1.2	0.6	0.4	0.7
35	Thymol	1290	2.5	1.3	0.9	1.8
36	Carvacrol	1299	0.2	0.5	0.6	0.8
37	Fragranyl acetate	1335	28.4	34.0	37.0	20.0
38	α-Terpinyl acetate	1352	0.6	3.4	5.1	1.1
39	Neryl acetate	1361	0.2	0.2	0.1	0.1
40	Decanoic acid	1369	–	–	0.7	0.3
41	E-Myrtanol acetate	1386	0.9	1.7	1.2	1.6
42	β-Caryophyllene	1419	1.2	1.2	0.9	1.5
43	Lavandulyl isobutanoate	1424	0.1	0.1	0.2	0.3
44	E-β-Farnesene	1456	Tr	0.1	Tr	-
45	Germacrene D	1485	1.6	1.1	1.9	1.5
46	β-Selinene	1490	–	0.4	0.3	0.5
47	Bicyclogermacrene	1500	0.6	0.9	0.9	0.5
48	Germacrene A	1509	0.7	0.2	–	0.5
49	Lavandulyl -2-methyl butanoate	1511	2.1	1.5	0.9	Tr
50	γ-Cadinene	1513	0.1	–	–	-
51	Dendrolasin	1571	1.5	1.2	0.8	2.1
52	Spathulenol	1578	–	0.1	0.6	0.3
53	Caryophyllene oxide	1583	1.2	1.2	1.4	3.2
54	10-epi-γ-eudesmol	1623	1.5	0.6	1.4	4.2
55	α-Muurolol	1646	1.1	1.9	2.1	3.6
56	β-Eudesmol	1650	1.1	–	0.1	0.2
57	Heptadecane	1700	0.4	0.2	0.1	0.3
58	Chamazulene	1731	0.4	0.3	0.2	0.3
59	E-β-Santalol acetate	1868	1.0	0.2	0.1	0.5
60	Hexadecanoic acid	1960	–	–	–	5.2

61	*n*-Eicosane	2000	–	–	–	4.2
62	Methyl linoleate	2085	–	–	–	3.1
63	*n*-Heneicosane	2100	–	–	–	1.5
64	*n*-Tricosane	2300	–	–	–	1.5
Number of identified compounds			53	48	50	59
Yield of the oil %			0.7	0.25	0.15	1.2
Monoterpene hydrocarbons			8.3	9.1	8.9	7.1
Oxygenated monoterpenes			72.4	77.2	76.0	55.1
Sesquiterpene hydrocarbons			4.7	4.3	3.0	4.5
Oxygenated sesquiterpenes			7.4	5.2	6.5	14.1
Others			3.3	2.2	1.9	16.5
Total identified			96.1	98.0	96.3	97.2

[a]Peak area of essential oil components; [b]KI: Kovats indices on HP-5 capillary column in reference to C_8-C_{28} *n*-alkanes[21]; [c]Components were identified on KI and GC-MS (gas chromatograph coupled with mass spectrometry and listed according to their elution on HP-5 MS capillary column (30 m); Tr = Trace < 0.1.

components[22]. The variations of the essential oil content and morphological values in ten accessions of *A. santolina* were collected from northwestern of Iran, have been investigated[23]. Two genotypes which were gathered from Lorestan and Kurdistan provinces, respectively, the highest mean of essential oil content (0.2 and 0.19 %, respectively). Considering the amount of essential oil in each genotype from each province, they have been mentioned that this feature varied with location with western Iran having the highest.

Essential oil variation among and within six *Achillea* species transferred from different ecological regions in Iran to the field conditions investigated[24]. According to the major compounds, four chemotypes were defined. Among them four *A. santolina* collected from Natanz, Malayer, Arak and Yasooj in center and west of Iran, were studied. Their yield essential oils were between 0.1-0.6 % and 43 components identified. comphor, borneol, bornyl acetate, germacrene D and spathulenol were major constituents.

In another experimental study, yarrow plant in late spring was collected from Sistan region, east of Iran, in 2008. The compounds of the essential oil were analyzed by GC/MS. In this study, camphor was the major compound of the essential oil[25].

According to Nemeth[26], studies within the last 15 years, an average of 54 compounds have been identified in different species. Among them, the largest numbers of components (149 compounds) were found in the oils of some *Achillea* species. 1,8-cineole, camphor, borneol, α-/β-pinenes are among the five most abundant monoterpene components. Among the monoterpenes, 1,8-cineole is major component in *Achillea* species[27-29]. In some *Achillea* species essential oil components, camphor and borneol are next ranks[30-33]. The previous studies reported that the major constituents of *A. tenuifolia* were γ-muurolene[34], limonene[35] and camphor[33,36,37].

Chamazulene, β-caryophyllene and oxide, eudesmol, α-bisabolol as well as its oxides and farnesene are the most frequently sesquiterpene constituents[26]. 1,8-Cineole, camphor, piperitone and ascaridole have reported by some researchers as the major constituent in several *Achillea* species in Turkey[32,38-40].

Conclusion

It could be concluded that difference in the essential oils and extract composition of *A. santolina* influenced by ecological conditions and type of the organ in which essential oil was produced and solvent that is used for extraction. The results of this study revealed the importance of comparing and exploring the variance of essential oils and extracts composition, from different parts of *A. santolina*, since this heterogeneous repartition of bioactive substances and classes of compounds between flower, leaf, stem and extracts entrained the variability of their potential antimicrobial activities.

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