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Comparison of terpene components from flowers of *Artemisia annua*

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Abstract

Terpene constituents of essential oils obtained by steam distillation from *Artemisia annua* flowers at the pre-, full- and post-flowering stage was investigated by gas chromatography (GC) and gas chromatography-mass spectrometric detector (GC-MS). The aim was to evaluate change the biosynthesis pathway of terpenes at different flowering stages. The samples studied showed that main components of essential oils were monoterpenes hydrocarbons (48.1%) and oxygenated monoterpenes (41%) in the pre-flowering, oxygenated monoterpenes (35.6%) and sesquiterpenes hydrocarbons (5.0%) in the full-flowering, and oxygenated monoterpenes (29.6%), sesquiterpenes hydrocarbons (32.2%) and oxygenated sesquiterpenes (25.3%) in the post-flowering, respectively. The relative content of monoterpenes decreased from pre-flowering to post-flowering, while that of sesquiterpenes increased. The results indicated that the biosynthesis pathway of terpenes might be changed at different flowering at stages, while the change of content and composition of terpenes might be a self-adaptation of *A. annua.*

Introduction

Artemisia annua L. is an annual herb native of China, where it has been used in the treatment of fever and malaria for many centuries. Many secondary metabolites of terpene peroxides were isolated from the plant, such as artemisia ketone, artemisinic alcohol, arteannuin B and myrcene hydroperoxide (Bertea et al., 2005; Brown et al., 2003). The most famous terpene peroxide is Artemisinin, which chemical structure is an amorphane-type sesquiterpene endoperoxide, and it has become an important plant-derived compound in the treatment of the chloroquine-resistant and cerebral malarias (Klayman, 1985). The essential oils, another important composition of *A. annua*, have been subjected to extensive chemical study. It was reported that the oils contained artemisia ketone, 1,8-cineole and camphor as key components. Significant variations in the percentage occurrence of different constituents have also been reported. The percentage of artemisia ketone, 1,8-cineole and camphor were reported to vary from 0.0 -63.0, 1.5-31.5 and 5.0-20.0%, respectively, Other major components reported were α-, β-pinene, borneol, carvacrol, thymol, myrcene, limonene, camphene, copaene, β -caryophyllene, α-terpineol, α-, β- and γ- elemene, sabinine, α-guaiene, caryophyllene, caryophyllene oxide, germacrene-D and so on (Fabien et al., 2002; Neetu et al., 2002; Perazzo et al., 2003; Soylu et al., 2005; Rasooli et al., 2003; Flora et al., 2004; Ma et al., 2007; Divya et al., 2007; Hashemi et al., 2007).

Chongqing, China, shares eighty percent of *A. annua* around the world, where an *A. annua* GAP Cultivation Demonstration Site has been built for three years. But

systemic study about the essential oils of the herb has not been previously mentioned in literature. The effect of flowering on terpenes content of *A. annua* flower essential oils at pre-, full- and post-flowering stage was investigated. In the present paper we report the analytical results of the essential oils at different flower developing stages.

Materials and Methods

A. annua cultivar Wuling-3938 plants were grown in the Artemisia GAP Cultivation Demonstration Site of Holleypharm, Chongqing, China. The flowers were harvested at pre-, full- and post-flowering stage in September to November 2007 on a same plant. The flowers were separated from other capitula organs, leaves and stem of *A. annua*, and identified by Mr. Rongchang Luo of Holley Natural Resource Exploiture Co. Ltd, Chongqing, China and deposited in the Herbarium, College of Bioengineering, Chongqing University, Chongqing, China.

Shade-dried plant materials (50.0 g) were hydro- distilled separately in Clevenger-type equipment for 4h. The oils were collected and dried over anhydrous sodium sulfate and stored in a refrigerator at 4°C for analysis.

GC analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with an FID and an HP-5 fused silica column (30 m × 0.32 mm i.d., 0.25 µm film thickness) with a 5% phenyl-substituted methylpolysiloxane phase. The oven temperature was programmed at 40°C for 4 min and then increased to 240°C at a rate of 4°C/min. Injector and detector temperatures were 250 and 265°C, respectively. The carrier gas, helium (99.999%), was adjusted to a linear velocity of 43 cm/s. The essential oil samples were diluted 5-fold, and 1 µL of a diluted solution was injected into the GC/MS in the split mode with a split ratio of 1/20.

MS analyses were performed using a Shimadzu MS-QP2010 with ionization energy of 70 eV, a scan time of 0.5 s and a mass range of 33–450 amu. The percentages of compounds were calculated by the area normalization method without considering response factors. The components of the oil were identified by comparison of their mass spectra with those of the spectrometer database using the NIST147 mass spectral database and also with those of authentic compounds. The identifications were confirmed by comparison of the fragmentation patterns and Retention index with those reported in the literature (Divya et al., 2007a; Flora et al., 2007; Divya et al., 2007b). The retention index was found with a standard mixture of C8 to C22 compounds under chromatography conditions, consistent with those of the chromatography conditions of the samples analyzed.

Results and Discussion

The flower essential oils were obtained from *A. annua* at pre-, full- and post-flowering stage with 2.2, 1.4 and 1.3% yield (relative to dried weight), respectively. Three oils were pale yellow, and the results of the analysis of the essential oils are given in Table I.

A total of sixty-three compounds were identified in three essential oil samples. The oils contain mainly monoterpenes and sesquiterpenes, representing 96.2, 98.9 and 99.3% of the oils in *A. annua* at pre-, full- and post-flowering stage, respectively. Monoterpenes hydrocarbons and oxygenated monoterpenes content was greater in the preliminary florescence oil, they were 48.1 and 41.0%. The main compounds in the oil were β-myrcene (37.7%), 1,8-cineole (16.1%) and camphor (15.0%). The oxygenated monoterpenes and sesquiterpenes hydrocarbons were dominants in the flourishing florescence oil (35.6 and 5.0%). The oil contained predominantly caryophyllene (19.4%), germacrene D (18.1%), camphor (15.8%), 1,8-cineole (10.6%), (Z)-β-farnesene (9.4%). The terminal florescence oil contains mainly oxygenated monoterpenes (29.6%), sesquiterpenes hydrocarbons (32.2%) and oxygenated sesquiterpenes (25.3%). The major constituents identified in the oil were camphor (16.6%), caryophyllene (16.3%), β-caryophyllene oxide (15.8%), β-farnesene (9.0%), (-)-spathulenol (7.2%). A significant difference was the absence of oxygenated sesquiterpenes in preliminary florescence, while the monoterpenes hydrocarbons were lacked in the terminal florescence oil.

It is reported (Liu et al., 1988) that the inflorescence oil of *A. annua* from Changchun, China, contained Artemisia ketone (63.1%), 1,8-cineole (1.5%), β-pinene (1.5%) and caryophyllene (1.9%). Another literature (Divya Goel et al., 2007) reported the major compounds of the cultivar *A. annua* petal oil were *trans*-sabinol (10.2%), paramentha-1, 4 (8)-dien-3-ol (10.1%) and 1,8-cineole (6.8%). The influence of transplanting time on inflorescence essential oil yield and composition has been studied (Flora Haider et al., 2007). Their result shows that oil yield was found to range from 0.5 to 1.6% (w/v). Camphor (23.30-57.00%), 1,8-cineole (5.60-21.40%) and β-caryophyllene (2.5-8.7%) were key compounds. The percentage occurrence of rest of the compounds was found to vary with different transplanting time. Our results were more similar to that of Flora Haider.

The content of the monoterpenes hydrocarbons were decreased sharply with flower developing, especially for β-myrcene, it from 37.7% in the preliminary florescence oil to 0.2% in the flourishing florescence oil, the compound was lacked in the terminal florescence oil (Figure 1A). The oxygenated sesquiterpenes were

Table I

Chemical composition of the flower essential oils at pre-, full- and post-flowering stage of *A. annua*

No	T_R	RIa	Components	Content (%)		
				Pre-flowering	Full-flowering	Post-flowering
1	5.0	816	(3-Methyl-2-oxiranyl)methanol	—	—	0.5
2	5.1	820	2-Ethoxypropane	—	1.7	0.5
3	7.3	928	Origanene	0.3	—	—
4	7.5	937	α-Pinene	0.9	—	—
5	7.8	955	Camphene	3.1	0.4	—
6	8.2	976	Sabinene	3.8	0.4	—
7	8.3	981	2,2-Dimethylhexanal	—	—	0.2
8	8.3	983	β-Pinene	1.5	—	—
9	8.5	991	β-Myrcene	37.7	0.2	—
10	8.6	995	Yomogi alcohol	—	—	0.7
11	8.6	995	2,3-Dehydro-1,8-cineole	—	0.6	—
12	9.1	1019	(+)-4-Carene	0.1	-	—
13	9.1	1021	No	—	0.1	—
14	9.3	1029	No	—	0.1	—
15	9.4	1032	Limonene	0.5	—	—
16	9.5	1037	1,8-cineole	16.1	10.6	0.3
17	9.9	1057	Artemisia ketone	0.1	0.2	2.4
18	9.9	1060	Tricyclene	0.3	—	—
19	10.0	1062	γ-Terpinen	—	0.3	—
20	10.3	1076	cis-β-Terpineol	0.4	0.5	—
21	10.4	1081	No	—	—	0.5
22	10.6	1092	5-(2-Methylenecyclopropyl)-1-pentanol	0.7	—	—
23	10.8	1100	(3E,5E)-2,6-Dimethyl-3,5,7-octatrien-2-ol	4.0	1.4	2.6
24	10.9	1104	No	—	0.2	—
25	10.9	1106	Nonanal	—	—	0.4
26	10.9	1107	Plinol C	0.6	0.6	—
27	11.4	1128	trans-p-Mentha-2,8-dienol	—	0.2	—
28	11.7	1140	ND	0.3	0.3	0.4
29	11.8	1143	Ipsdienol	—	—	0.4
30	11.9	1149	Pinocarveol	0.3	0.2	0.4
31	12.0	1152	Berbenol	0.2	—	—
32	12.1	1157	Camphor	15.0	15.8	16.6
33	12.3	1165	Nerol	0.3	—	—
34	12.3	1165	Lavandulol	—	—	0.4
35	12.3	1167	(-)-cis-Myrtanol	—	0.2	—
36	12.3	1168	Isogeraniol	—	0.5	0.2
37	12.4	1171	ND	—	—	0.2
38	12.5	1176	Myrcenol	0.2	0.4	—
39	12.6	1180	Borneol	0.5	1.1	3.9

Table I

Chemical composition of the flower essential oils at pre-, full- and post-flowering stage of *A. annua* (Cont.)

No	T_R	RIa	Components	Content (%)		
				Pre-flowering	Full-flowering	Post-flowering
40	12.8	1187	4-Terpineol	0.6	1.2	1.0
41	12.9	1194	iso-Amyl tiglate	0.5	0.5	0.4
42	13.0	1199	1,5-Menthadien-7-ol	—	0.1	—
43	13.1	1201	α-Terpineol	1.3	0.3	0.2
44	13.2	1204	Myrtenol	0.5	0.2	—
45	13.5	1217	trans-3(10)-Caren-2-ol	0.2	0.3	0.5
46	13.9	1234	(E)-3(10)-Caren-4-ol	—	0.2	—
47	14.3	1247	(2E)-2,7-Dimethyl-2,6-octadien-1-ol	0.1	0.1	—
48	14.3	1248	ND	—	—	0.2
49	14.6	1259	4,6,6-Trimethylbicyclo[3.1.1]hept-3-en-2- yl acetate	—	1.6	—
50	15.1	1279	Nerol acetate	0.2	—	0.4
51	16.0	1310	Hydroxy-α-terpenyl acetate	—	0.6	—
52	18.6	1377	Copaene	—	1.1	1.4
53	19.2	1392	β-Elemen	—	—	1.5
54	20.8	1420	β-Caryophyllene	2.3	19.4	16.3
55	22.4	1445	β-Farnesene	2.6	9.4	9.1
56	22.9	1454	α-Caryophyllene	—	1.0	—
57	23.9	1470	Chamigren	1.8	—	—
58	24.5	1479	Germacrene D	1.9	18.1	4.0
59	25.5	1495	γ-Elemene	0.3	—	—
60	25.7	1498	Germacrene B	—	0.9	—
61	33.1	1570	(-)-Spathulenol	—	1.8	7.2
62	33.6	1575	β-Caryophyllene oxide	—	3.0	15.8
63	47.7	1681	Aromadendrene oxide-(2)	—	2.8	2.2
64	62.9	1904	ND	—	—	1.4
65	63.0	1907	δ-Cadinol	—	1.1	—
66	63.5	1919	(10Z,12Z)-9-Methyl-10,12-hexadecadienyl acetate	—	—	1.1
67	64.4	1942	ND	0.4	—	—
68	65.2	1961	ND	—	—	0.8
69	65.4	1967	n-Hexadecanoic acid	—	—	4.4
70	65.8	1977	ND	0.3	—	—
71	65.9	1979	9,12,15-Octadecatrienal	—	—	0.3
72	70.6	2106	trans-Phytol	—	—	0.4
73	71.4	2131	Stearolic acid	—	—	0.5
74	76.9	2297	2,6,10,14-Tetramethylheptadecane	—	—	0.6
75	Total identified			96.2		
76	Monoterpenes hydrocarbons			—		
77	Oxygenated monoterpenes			29.6		
78	Sesquiterpenes hydrocarbons			32.2		
79	Oxygenated sesquiterpenes			25.3		
80	Fatty acids and aliphatic esters			7.2		

TR = retention time; ND = not identified; (-) Not detected; aRI is the Retention index relative to C8-C22 n-alkanes on the HP-5ms column

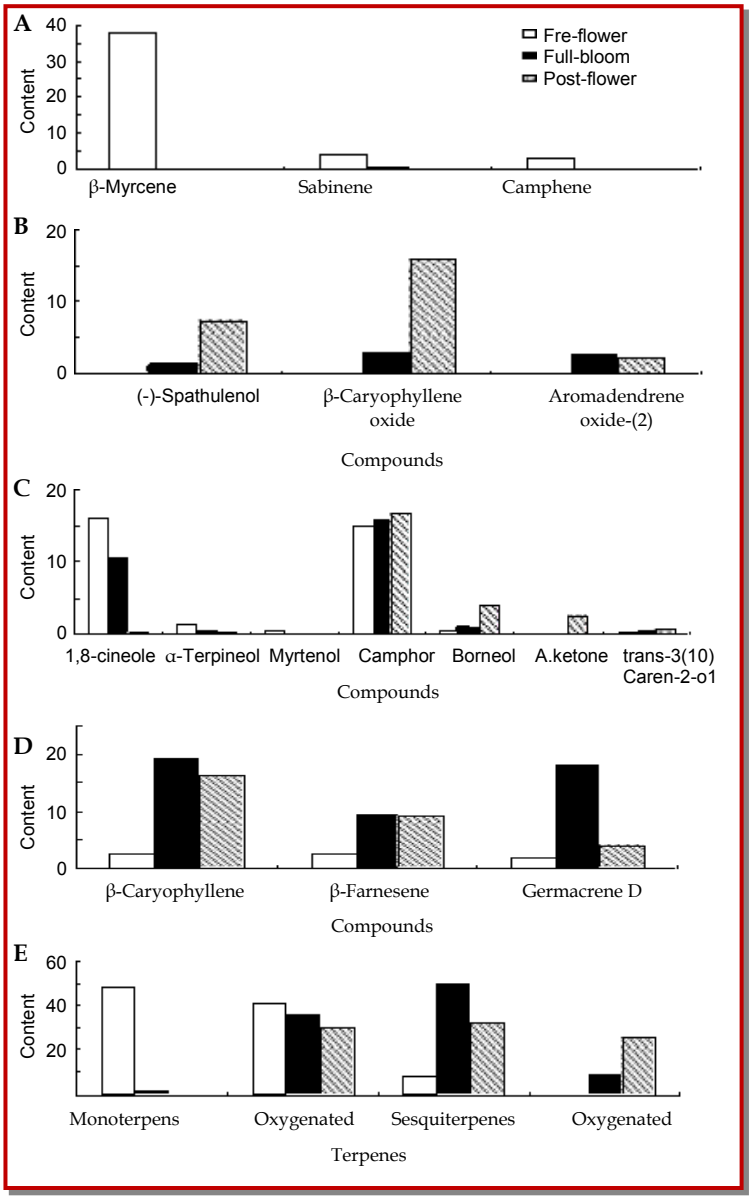

Figure 1: Content of monoterpene hydrocarbons (A), oxygenated sesquiterpenes (B), oxygenated monoterpenes (C), sesquiterpene hydrocarbons (D) and terpenes (E) of *A. annua* flower at different flowering stages

increased quickly with flower developing, the (-)-spathulenol and β-caryphyllene oxide were nice examples, both the compounds weren't detected in the preliminary florescence oil, there were only 1.8% and 3.0% in the flourishing florescence oil, they were added rapidly to 7.2 and 15.8% in the terminal florescence oil (Figure 1B).

In general, the oxygenated monoterpenes were declined slowly with flower developing, among major compounds, artemisia ketone, camphor, borneol and *trans*-3 (10)-Caren-2-ol were raised tendency, while 1,8- cineole was declined (Figure 1c). Interestingly, the sesquiterpenes hydrocarbons increased sharply from 8.9% at pre-flowering stage to 5.0% (at the top of content) at full -flowering stage, followly by, which decreased slowly to 32.2% at post-flowering stage. Among of these compounds, the change of β- caryophyllene and β-farnesene was good examples. The content of former from 2.3% added to 19.4%, then decreased to 16.3%; and that of the later from 2.6% added to 9.4%, then decreased to 9.0% (Figure 1D).

As to the change of all terpenes in flower oils of *A. annua* at different flowering stage, the content of the monoterpenes hydrocarbons and oxygenated monoterpenes were the highest at the pre-flowering stage, and the sesquiterpenes hydrocarbons arrive to the top

at the full-flowering stage, while the oxygenated sesquiterpenes in flower oils were peak at the post-flowering phase (Figure 1E).

The present results have shown that the content of terpenes in the *A annua* oils has close relationship with flower developing. The flowering effect on monoterpenes content of plant oil has been reported elsewhere (Dudai et al., 1992). So flowering may be change the biosynthesis pathway of terpenes in the *A. annua* flower oil, in turn, the change of content of terpenes in the oils may be the self-adaptation for the physiological phenomenon of flowering.

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References

Bertea CM, Freije JR, Van DWH, Verstappen FW, Perk L, Marquez V, De Kraker JW, Posthumus MA, Jansen BJ, de Groot A, Franssen MC, Bouwmeester HJ. Identification of intermediates and enzymes involved in the early steps of artemisinin biosynthesis in *Artemisia annua*. Planta Med. 2005; 71: 40-47.

Brown GD, Liang GY, Sy LK. Terpenoids from the seeds of *Artemisia annua* L. Phytochemistry 2003; 64: 303-23.

Dudai N, Putievsky E, Ravid U, Palevitch D, Halevy AH. Monoterpene content in *Origanum syriacum* as effected by environmental conditions and flowering. Phys Plant. 1992; 84: 453–59.

Goel D, Singh V, Ali M, Mallavarupu GR, Kumar S. Essential oils of petal, leaf and stem of the antimalarial plant *Artemisia annua*. J Nat Med. 2007a; 61: 187-91.

Goel D, Singh V, Ali M, Mallavarupu GR, Kumar S Composition of the essential oil from the root of *Artemisia annua*. J Nat Med. 2007b; 61: 458-61.

Haider F, Dwivedi P, S Singh, Naqvi AA, Bagchi G. Influence of transplanting time on essential oil yield and composition in *Artemisia annua* plants growth under the climatic conditions of sub-tropical north India. Flavour Fragrance J. 2004; 19: 51-53.

Hashemi P, Abolghasemi MM, Fakhari AR, Ebrahimi SN, Ahmadi S Hydrodistillation–solvent microextraction and GC –MS identification of volatile components of *Artemisia aucheri*. Chromatographia 2007; 66: 283-86.

Iraj Ri, Rezaee MB, Moosavi ML, Kamkar J. Microbial sensitivity to and chemical properties of the essential oil of *artemisia annua* L. J Essent Oil Res. 2003; 15: 59-62.

Jain N, Srivastava SK, Aggarwal KK, Kumar S, Syamasundar KV, Jain N, Kumar S. Essential oil composition of *Artemisia annua* L. 'Asha' from the plains of Northern India. J Essent Oil Res. 2002; 14: 305-07.

Juteau F, Masotti V, Bessière JM, Dherbomez M, Viano J Antibacterial and anti-oxidant activities of *Artemisia annua* essential oil. Fitoterapia 2002; 73: 532-35.

Klayman DL. Qinghaosu (Artemisinin): An antimalarial drug from China. Science 1985; 228: 1049-55.

Liu Q, Yang ZY, Deng ZB, Sa GH, Wang XJ. Preliminary analysis on chemical constituents of essential oil from inflorescence of *A. annua L.* Acta Botanica Sinica 1988; 30: 223 -25

Ma CF, Wang HH, Lu X, Li HF, Liu B, Xu GW. Analysis of *Artemisia annua* L. volatile oil by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. J Chromatogr. A 2007; 1150: 50–53.

Perazzo FF, Carvalho JCT, Carvalho JE, Rehder VLG. Central properties of the essential oil and the crude ethanol extract from aerial parts of *Artemisia annua* L. Pharmacol Res. 2003; 48: 497-502.

Soylu EM, Yigitbas H, Tok FM, Soylu S, Kurt S, Baysal Ö, Kaya AD. Chemical composition and antifungal activity of the essential oil of *Artemisia annua* L. against foliar and soil-borne fungal pathogens. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz. 2005; 112: 229-39.

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