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Chemical variation in the essential oil of *Ephedra sinica* from Northeastern China

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Abstract

Hydro-distillated volatile oils of *Ephedra sinica* Stapf. from six populations of Inner Mongolia in Northeastern China were analyzed by using GC/MS. Ninety-nine compounds were identified in the oils and a relatively high variation in their contents was found. The main constituents of the essential oils were α-terpineol (19.28–52.23%), *p*-vinylanisole (0.59–11.64%), 3-methyl-2-buten-1-ol (0–5.44%), tetramethylpyrazine (0.63–8.99%), terpine-4-ol (1.17–4.37%), α-linalool (1.62–5.15%), phytol (1.24–15.73%), γ-eudesmol (0–7.77%), and eudesm-7(11)-en-4-ol (0.41–6.13). Six populations were divided into two chemotypes based on cluster analysis and principal component analysis (PCA); one rich in α-terpineol and *p*-vinylanisole, and the other rich in phytol, γ-eudesmol, and eudesm-7(11)-en-4-ol.

Keywords: *Ephedra sinica*; Essential oils; GC/MS; Chemotype; Northeastern China

1. Introduction

The family *Ephedraceae* consists of about 50 species in the world (Price, 1996). Most of these species are distributed in temperate and subtropical regions of Asia, Europe, and North and Central Americas. The genus *Ephedra* consists of a group of perennial and dioecious shrubby plants growing up to 4 feet tall, with slender and joined stems. Their leaves are reduced to scales and grow in opposite pairs or whorls of three. They usually grow on plains, sandy soil, dry slopes, and dry mountain sides.

Many *Ephedra* species, e.g., *Ephedra nevadensis*, *Ephedra trifurca*, *Ephedra geradinia*, and *Ephedra sinica*, have been used as important medicinal herbs worldwide for a long time. In particular, *E. nevadensis* and *E. trifurca* grown in southwest America were used to make ''Mormon tea'', ''joint-fir'', or ''Squaw tea''. They were brewed by the natives not only to treat allergies and other cold symptoms, but also as a stimulant. In India and Pakistan, *E. geradinia* was used to produce a special drink called ''soma'', which was believed to promote longevity and its stems were used to treat asthma. *E. sinica*, also known as ''Mahuang'', is an endemic species of Mongolian region and one of the most famous medicinal plants in the world. In Inner Mongolia of Northeastern China, the annual production of *E. sinica* has reached more than 100,000 tons. Most of the raw

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material of *Ephedra* extracts in the world come from this region.

Historically, *E. sinica* has been used as an important medicinal herb in China for more than 5000 years. During the period of the ancient Chinese Han Dynasty, *E. sinica* had been used to treat the common cold, asthma, bronchitis, and arthritis. The most common preparation of *E. sinica* was as a tea. Its stems were dried in the sun and cut into pieces, boiled in water for about thirty minutes, and the liquid extract was then consumed. In the last century, *E. sinica* became a most popular plant for its extract was used as central nervous system (CNS) stimulants and mood enhancers as well as dietary supplements in various health foods in the western countries (Gurley, Wang, & Gardner, 1998), which are claimed to be effective for weight-loss or as energy booster. In the past decade, however, more and more evidence suggested that the misuses or abuses of the herb could cause possible hazards to public health, even lead to cardiovascular symptoms, stroke, and death (Haller & Benowitz, 2000; Zaacks, Klein, Tan, Rodriguez, & Leikin, 1999). In February 2004, after an intense debate, the United States Food and Drug Administration (FDA) announced to ban the retail sales of dietary supplements containing any of the ephedra alkaloids. Despite of this setback in use in the United States, *E. sinica* remains to be one of the most important medicinal herbs, especially in China.

Although the pharmacological activities exerted by *E. sinica* are believed to come primarily from ephedrine-like alkaloids, other types of constituents such as essential oils are medicinal as well. For example, 2,3,5,6-tetramethylpyrazine and terpineol were found in the essential oils of *E. sinica* (Miyazawa, Minamino, & Kameoka, 1997). The former is a kind of cardiovascular drug (Liu et al., 1990) and the latter is an important raw material in pharmaceutical industry. Few studies on chemistry of the essential oils of *E. sinica* were carried out (Miyazawa et al., 1997) and athough the main constituents of this species were reported as α-terpineol (13.0%), tetramethylpyrazine (3.9%), terpinen-4-ol (3.9%), linalool (3.2%), 2,3-dihydro-2-methylbenzo-furan (3.1%) and *cis-p*-menth-2-en-7-ol (3.1%).

As a class of compounds in plants with important functions in the defense against pathogenic fungi and attraction of pollinators, the compositions of the essential oils from many plants such as *Melaleuca alternifolia*, *Hyptis crenata*, and *Jasminum sambac*, are closely related to their genotypes and growing environments (Lee et al., 2002; Rao & Rout, 2003; Zoghbi et al., 2002). However, for a small number of plants, there is no significant difference among different populations. For example, three populations of *Satureja sahendica* collected from three different provinces in Northwestern and Western Iran belonged to one chemotype and their oil composition was similar to that of *Satureja bachtiarica* and *Satureja spicigera* (Sefidkon, Jamzad, & Mirza, 2004). Although the main constituents of *E. sinica* were reported, few studies on chemistry of the essential oils were carried out (Miyazawa et al., 1997), nor chemical variation in the essential oils of *Ephedra sinica* has been investigated. In this study, we collected six *E. sinica* populations from east to west Inner Mongolia of Northeastern China at the end of September, 2004, when *E. sinica* was thought to be of the best quality by Chinese traditional pharmacologists, for determining the constituents of the essential oils and evaluating population variation of the oils.

2. Materials and methods

2.1. Plant materials

Samples of *E. sinica* were collected at the end of September, 2004 from six locations in Inner Mongolia, China (Fig. 1): Chifeng-Wusan (N: 42°15′; E: 118°47′), Chifeng-Sanyanjing (N: 42°14′; E: 118°55′), Zhengxiangbaiqi (N: 42°15′; E: 114°56′), Hohhot (N: 40°23′; E: 111°54′), Baotou (N: 40°52′; E: 110°04′), and Ertokeqi (N: 39°05′; E: 107°58′). All six populations grew on dry slopes with similar environments and no obvious morphological variation was found among them. These samples were identified and voucher specimens were deposited in the MOE Laboratory for Biodiversity Science and Ecological Engineering, Fudan University. The aerial parts of all samples were dried and stored at room temperature and subsequently cut into small pieces for distillation of their essential oils.

2.2. Isolation of the essential oils

The aerial parts of the six samples, i.e., Chifeng-Wusan (600 g), Chifeng-Sanyanjing (540 g), Zhengxiangbaiqi (325 g), Hohhot (525 g), Baotou (410 g), and Ertokeqi (480 g), were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. Each sample was repeated for four times. The essential oils were collected and then stored with anhydrous sodium sulfate in Eppendorf tubes at 4 °C until analyzed and tested.

2.3. Analysis of the oils

The essential oils from the aerial parts of *E. sinica* were conducted as previously described (Nan et al., 2003). Briefly, GC analyses were carried out on a HP-6890 gas chromatography equipped with a FID and a HP-5 capillary column (30 m in length, 0.25 mm in diameter, 0.25 μm in film thickness) using N_2 as carrier gas (1 ml/min). A 1 μl aliquot of oil was injected into the column using a 10:1 split injection, with temperature set at 250 °C. The GC program was initiated by a column

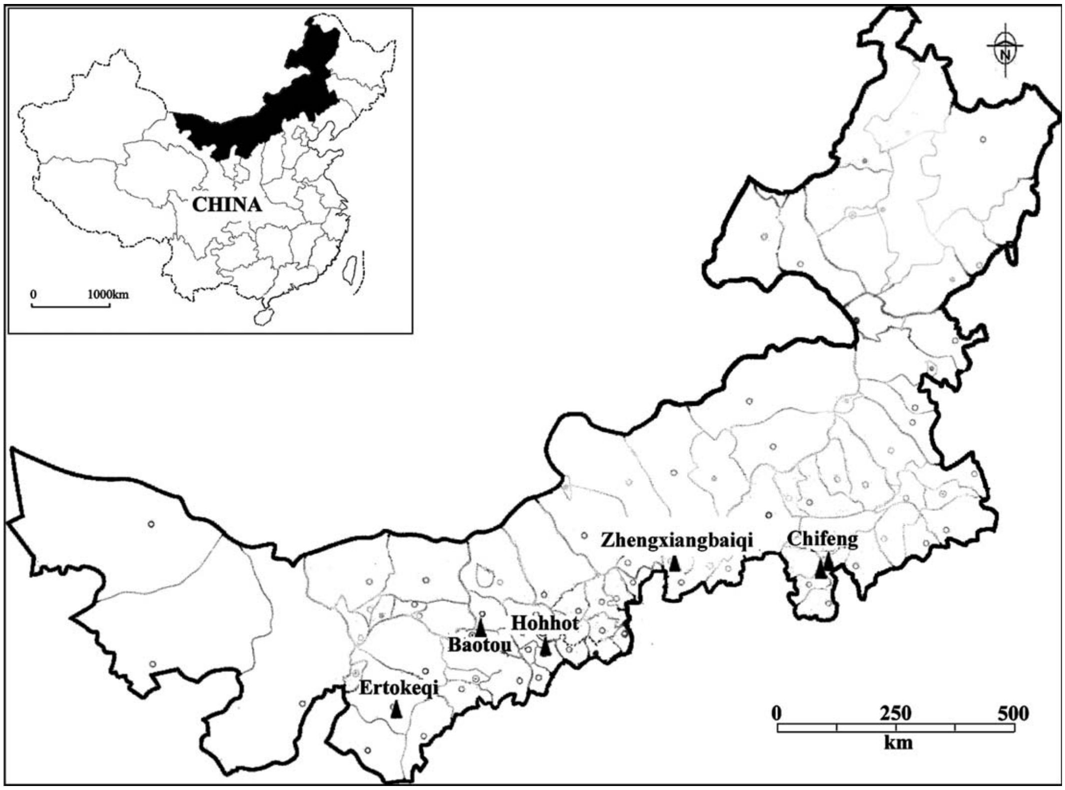

Fig. 1. Six sampling locations of *Ephedra sinica* in Inner Mongolia of Northeastern China.

temperature set at 60 °C for 2 min, increased to 250 °C at a rate of 10 °C/min, and held for 10 min.

GC/MS analyses were performed on a combined GC/MS instrument (Finnigan Voyager, San Jose, CA, USA) with a HP-5 fused silica capillary column (30 m in length, 0.25 mm in diameter, 0.25 μm in film thickness) using He as carrier gas (1 ml/min). The mass spectrometer was operated in the 70 eV EI mode with scanning from 41 to 450 amu at 0.5 s, and mass source was set at 200 °C. The identifications of the volatile constituents were based on GC retention indices (relative to *n*-alkanes, from C8 to C20) and computer matching of their mass spectral fragmentation patterns with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998).

2.4. Statistical analyses

To reveal the relationship among the six populations sampled based on compositions of essential oils and to identify the possible constituents, which are the determinate chemotypical factors of *E. sinica*, the composition data matrix of six populations was analyzed using principal component analysis (PCA) with SPSS version 12.0. Cluster analysis of the six populations based on Euclidean distances was performed with the unweighted pair-group method using an arithmetic average (UPGMA) with the NTSYSpc version 2.02.

3. Results and discussion

Hydrodistillation of six populations of *E. sinica* yielded from 0.02% to 0.05% dry wt (v/w) yellowish oils. The highest oil content was found in the sample from Ertokeqi (0.05%), followed by Baotou (0.05%), Chifeng-Wusan (0.04%), Zhengxiangbaiqi (0.03%), Hohhot (0.03%), and Chifeng-Sanyanjing (0.02%). The relative contents of the oils seem to be low, but 3–8 times higher than those previously reported (Miyazawa et al., 1997). Considering the substantial annual production of *E. sinica* and the readily available raw materials, the low contents of essential oils may be acceptable. The chemical constituents identified by GC/MS in combination with retention indices (RI) in the essential oil and their percentages are listed in Table 1 according to their elution order.

Ninety-nine compounds were identified in the six sample oils, accounting for 97.38–98.42% of the oils. The essential oils in the samples from Chifeng-Wusan, Chifeng-Sanyanjing, Zhengxiangbaiqi, Hohhot, Baotou, and Ertokeqi were composed of 65, 61, 74, 77, 83, and 81 identified compounds accounting for

Table 1
Constituents of the essential oils of *Ephedra sinica* from six populations from Inner Mongolia of Northeastern China

	Compound	RI	Chifeng-Wusan (%)	Chifeng-Sanyanjing (%)	Zhengxiangbaiqi (%)	Hohhot (%)	Baotou (%)	Ertokeqi (%)
1	3-Methyl-2-buten-1-ol		5.44	tr	–	–	tr	tr
2	Bicyclo[4.1.0]hept-2-ene		–	tr	–	tr	tr	tr
3	Hexanal	798	tr	–	–	–	tr	–
4	2-Hexen-1-al	854	tr	tr	tr	–	tr	–
5	(*Z*)-Hex-3-en-1-ol	857	0.12	0.20	0.10	tr	tr	tr
6	Cyclohexanol	870	–	–	–	–	–	tr
7	1-Hexanol	872	–	–	–	–	–	tr
8	Styrene	895	–	–	–	–	–	tr
9	Benzaldehyde	965	0.40	0.12	0.17	0.20	0.30	0.28
10	2-Methyl-oct-1-en-3-yne	981	0.32	0.15	tr	0.20	0.52	0.91
11	Myrcene	994	–	–	–	–	–	tr
12	α-Phellandrene	1009	–	–	–	–	–	tr
13	4-Carene	1022	0.34	tr	tr	tr	0.19	0.30
14	*p*-Cymene	1030	0.24	0.12	tr	0.10	0.30	–
15	D-Limonen	1035	1.38	0.27	tr	0.36	1.13	1.76
16	*trans*-β-Ocimene	1042	–	–	–	–	–	tr
17	*cis*-β-Ocimene	1053	tr	0.34	tr	0.17	0.11	1.04
18	γ-Terpinene	1065	0.41	0.12	tr	0.18	0.28	0.68
19	*cis*-Linalool oxide	1079	–	–	–	–	–	0.10
20	Tetramethylpyrazine	1090	8.99	0.71	3.98	3.48	1.97	0.63
21	Terpinolene	1094	1.93	0.64	0.10	0.81	1.30	3.14
22	Methyl benzoate	1098	–	–	–	–	–	0.20
23	α-Linalool	1103	2.08	3.03	1.62	2.80	2.31	5.15
24	Nonanal	1107	0.11	0.10	tr	0.15	0.11	0.13
25	*cis*-Menth-2-en-1-ol	1130	0.11	0.12	tr	0.15	0.13	0.11
26	Terpinen-1-ol	1142	0.17	-	tr	tr	0.13	0.12
27	β-Terpineol	1153	1.13	0.46	0.13	0.47	0.64	0.62
28	*p*-Vinylanisole	1160	1.55	11.64	0.59	5.34	3.81	13.74
29	(*E*)-2-Nonenal	1165	–	–	–	tr	tr	–
30	Isoborneol	1172	–	–	–	–	–	0.21
31	Acetylbenzoyl	1175	0.22	0.73	1.14	0.50	1.91	0.49
32	Ethyl benzoate	1176	–	–	–	–	–	0.27
33	Terpine-4-ol	1186	3.03	3.10	1.17	2.92	4.37	4.00
34	*p*-Cymen-8-ol	1193	0.20	–	0.14	0.17	0.17	tr
35	α-Terpineol	1201	47.95	52.23	19.28	40.18	36.56	43.33
36	Dihydrocarveol	1206	2.06	0.85	0.60	2.50	1.74	1.29
37	*trans*-Piperitol	1216	–	–	tr	0.12	tr	tr
38	Citronellol	1233	0.77	0.90	1.06	1.22	2.17	0.80
39	Cumin aldehyde	1250	–	0.14	tr	0.21	0.11	–
40	Geraniol	1260	1.34	2.61	5.02	4.01	2.93	4.56
41	*trans*-*p*-Menth-2-en-7-ol	1268	2.31	2.97	0.82	4.81	2.19	1.35
42	Geranial	1275	–	–	–	–	–	tr
43	*cis*-*p*-Menth-2-en-7-ol	1275	tr	–	0.10	0.11	0.13	0.21
44	Perilla aldehyde	1286	0.83	1.37	0.38	1.58	1.21	0.82
45	α-Terpine-7-al	1294	0.20	0.21	tr	0.19	0.13	0.19
46	*p*-Cymen-7-ol	1296	–	tr	tr	0.14	0.10	tr
47	Perrilla alcohol	1307	0.11	0.13	0.12	0.20	0.13	–
48	2,4-Decadienal	1323	–	–	tr	0.14	0.12	tr
49	Eugenol	1371	0.20	0.23	0.25	0.28	0.41	0.27
50	β-Damascenone	1394	–	0.49	tr	0.77	0.37	tr
51	*n*-Tetradecane	1403	–	–	0.12	–	–	–
52	Hexahydropseudoionone	1408	0.29	0.24	tr	0.12	0.19	–
53	Longifolene	1418	–	–	–	–	–	tr
54	α-Bergamotene	1449	tr	–	–	0.20	0.20	0.49
55	Geranyl acetone	1460	0.11	–	–	0.43	0.29	0.15
56	Patchoulene	1467	–	–	tr	–	–	tr
57	Aromadendrene	1481	0.31	0.34	0.20	0.73	0.87	0.57
58	γ- Muurolene	1494	–	–	tr	0.26	0.13	0.18
59	β-Ionone	1498	0.33	0.52	0.74	1.54	0.67	0.38
60	δ-Cadinene	1540	0.16	0.16	tr	0.33	0.19	0.15
61	Guaia-3,9-diene	1556	0.18	0.25	0.38	0.19	0.17	0.39

(*continued on next page*)

Table 1 (*continued*)

	Compound	RI	Chifeng-Wusan (%)	Chifeng-Sanyanjing (%)	Zhengxiangbaiqi (%)	Hohhot (%)	Baotou (%)	Ertokeqi (%)
62	*n*-Dodecanoic acid	1568	–	–	1.36	0.23	0.64	–
63	Hexenyl benzoate	1584	0.17	2.16	0.63	1.63	1.31	0.87
64	Hexyl benzoate	1589	–	0.16	0.14	0.10	0.12	tr
65	Spathulenol	1595	–	–	–	–	–	tr
66	Hexadecane	1604	0.42	0.38	0.80	0.46	0.53	–
67	Guaiol	1618	0.36	0.30	0.44	0.70	0.80	0.35
68	γ-Eudesmol	1646	0.93	–	7.77	0.58	0.47	0.82
69	β-Eudesmol	1654	0.30	0.25	1.64	0.46	0.80	0.25
70	*t*-Muurolol	1666	0.49	–	2.01	1.36	0.62	0.15
71	α-Eudesmol	1677	0.19	–	1.36	0.58	0.89	0.18
72	Bulnesol	1690	0.41	0.33	2.47	0.42	0.46	0.25
73	Cadalene	1696	–	–	0.28	0.11	0.11	tr
74	*n*-Heptadecane	1705	0.40	0.13	0.82	0.39	0.93	–
75	Eudesm-7(11)-en-4-ol	1722	0.53	0.57	6.13	0.56	0.41	0.51
76	Farnesol	1751	0.23	–	–	–	0.13	tr
77	Myristic acid	1765	–	–	–	tr	0.19	0.18
78	Benzyl benzoate	1784	–	0.15	0.34	0.13	0.17	tr
79	Cyclocolorenone	1791	0.96	0.65	3.55	0.49	0.30	0.93
80	*n*−Octadecane	1806	0.29	0.11	0.73	0.35	0.56	–
81	(*E*,*E*)-Farnesyl acetate	1816	tr	–	0.25	tr	0.29	–
82	Hexahydrofarnesyl acetone	1854	0.42	0.53	1.76	0.61	1.74	0.34
83	(*Z*)-3-Hexenyl cinnamic ester	1897	–	0.29	–	0.15	0.25	tr
84	*n*-Nonadecane	1902	0.18	–	0.50	0.21	0.26	–
85	Isophytol	1959	–	–	tr	tr	tr	–
86	*n*-Hexadecanoic acid	1969	2.17	0.16	3.71	2.25	3.38	0.35
87	Geranyl benzoate	1978	–	0.89	–	–	–	tr
88	Ethyl hexadecanoate		–	0.12	tr	–	0.17	–
89	*n*-Heneicosane		0.13	0.12	0.48	0.13	0.68	tr
90	Phytol		3.29	4.07	15.73	4.84	6.87	1.24
91	Methyl linolenic ester		0.47	0.26	–	tr	0.72	tr
92	*n*-Docosane		tr	–	–	tr	0.18	tr
93	*n*-Octadecanol		–	–	0.11	tr	tr	–
94	*n*-Tricosane		0.10	0.18	1.07	0.39	0.48	0.18
95	*n*-Tetracosane		0.10	0.26	0.85	0.40	0.46	0.12
96	*n*-Pentacosane		0.10	0.27	1.25	0.49	0.44	0.13
97	*n*-Hexacosane		tr	0.17	0.42	0.18	0.26	tr
98	*n*-Heptacosane		tr	0.21	1.12	0.33	0.51	0.13
99	*n*-Nonacosane		–	–	1.14	–	0.51	–
Monoterpene compounds			79.01	85.2	37.79	76.21	68.78	86.92
Sesquiterpene compounds			6.28	4.6	28.3	10.01	9.07	7.5
Alkanes and fatty acids			4.03	2.71	14.21	6.49	10.05	1.19

% Percentage of the content of each constituent in total essential oil ($n = 4$). RI = retention index; tr = traces quantities (<0.1).

98.57%, 98.42%, 98.07%, 97.38%, 97.89%, and 97.72% of the whole oil, respectively. The main constituents in the oils from Chifeng-Wusan were α-terpineol (47.95%), tetramethylpyrazine (8.99%), 3-methyl-2-buten-1-ol (5.44%), phytol (3.29%), and terpine-4-ol (3.03%); in the oils from Chifeng-Sanyanjing were α-terpineol (52.23%), *p*-vinylanisole (11.64%), phytol (4.07%), terpine-4-ol (3.10%), and α-linalool (3.03%); in the oils from Zhengxiangbaiqi were α-terpineol (19.28%), phytol (15.73%), γ-eudesmol (7.77%), eudesm-7(11)-en-4-ol (6.13%), geraniol (5.02%), tetramethylpyrazine (3.98%), *n*-hexadecanoic acid (3.71%), and cyclocolorenone (3.55%); in the oils from Hohhot were α-terpineol (40.18%), *p*-vinylanisole (5.34%), phytol (4.84%), *trans*-*p*-menth-2-en-7-ol (4.81), geraniol (4.01%), and tetramethylpyrazine (3.48%); in the oils from Baotou were α-terpineol (36.56%), phytol (6.87%), terpine-4-ol (4.37%), *p*-vinylanisole (3.81%), and *n*-hexadecanoic acid (3.38%); and in the oils from Ertokeqi were α-terpineol (43.33%), *p*-vinylanisole (13.74%), α-linalool (5.15%), geraniol (4.56%), terpine-4-ol (4.00%), and terpinolene (3.14%). Except the oils from Zhengxiangbaiqi, the oils from other populations are dominated by monoterpenes, while the contents of sesquiterpenes and fatty acids in the oils from Zhengxiangbaiqi are relatively high. Compared with the previous report (Miyazawa et al., 1997), the content of α-terpineol in our samples was much higher. Fig. 2 showed the comparison between the percentages of some constituents in the oils.

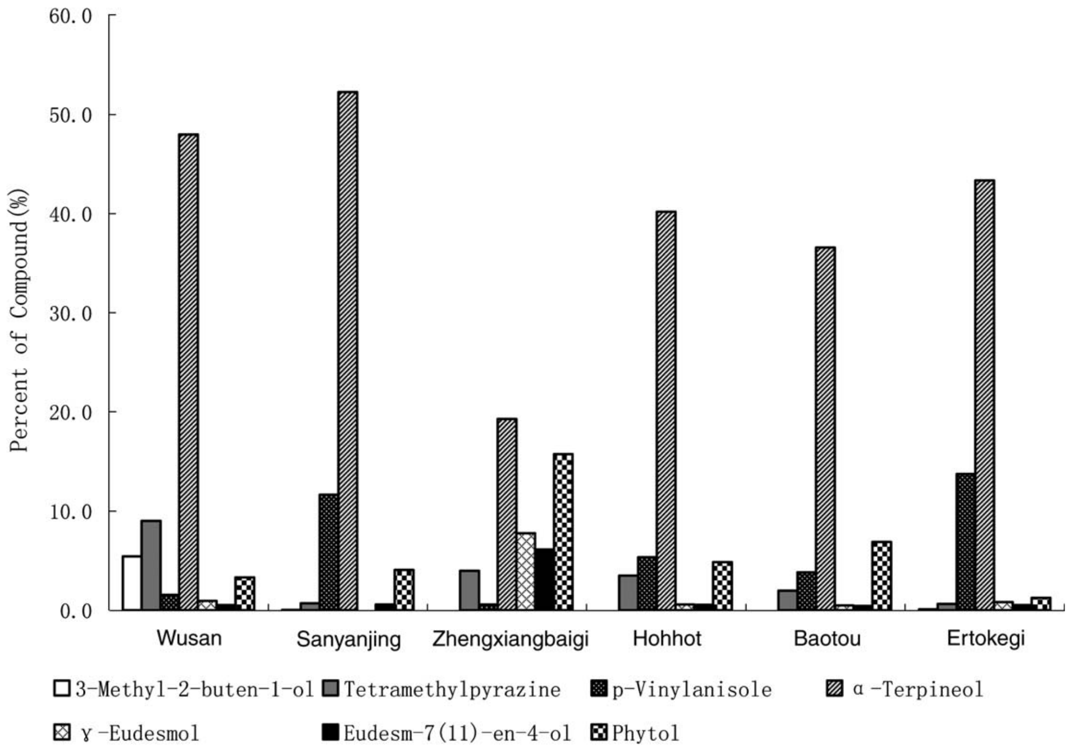

Fig. 2. Comparison of seven major components of the essential oils of *Ephedra sinica*.

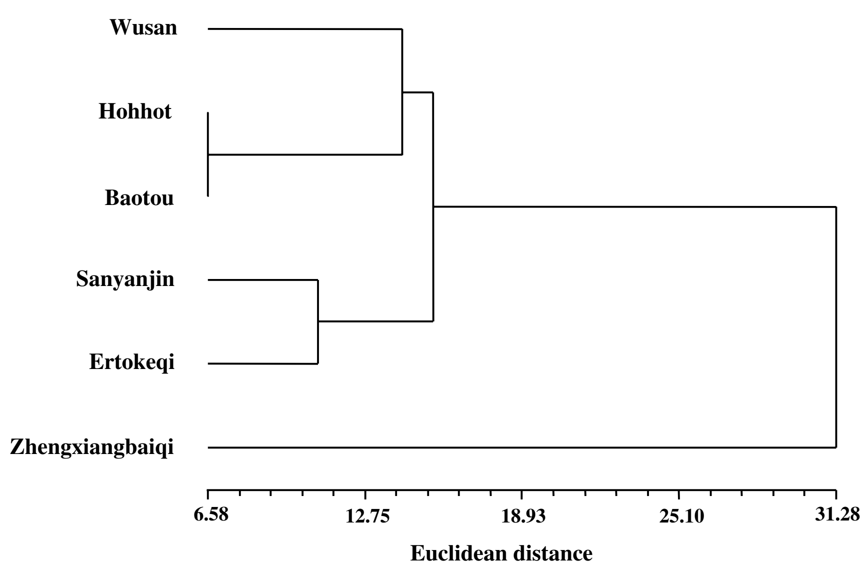

Fig. 3. A dendrogram of six populations of *Ephedra sinica* in Inner Mongolia of Northeastern China based on Euclidean distances and UPGMA clustering method.

In order to determine and verify the variations of essential oils in different populations of *E. sinica*, the composition data were analyzed by cluster analysis and PCA. Cluster analysis generated a dendrogram shown in Fig. 3. The results show that the population from Zhengxiangbaiqi is well separated from others, and two neighbor populations from Chifeng are separated, while variation between populations from Baotou and Hohhot is minor. No significant association was found between the essential oil composition and geographical distribution of the six populations. The 2D graphical representation of principal component analysis is shown in Fig. 4 and contains 98% of the information of the original data. It is obvious that the population from Zhengxiangbaiqi is quite different from others in term of chemical compositions in essential oils.

Both methods suggested that the population from Zhengxiangbaiqi possessed a different chemotype from the other five investigated in this study. The population from Zhengxiangbaiqi, rich in sesquiterpenes and fatty

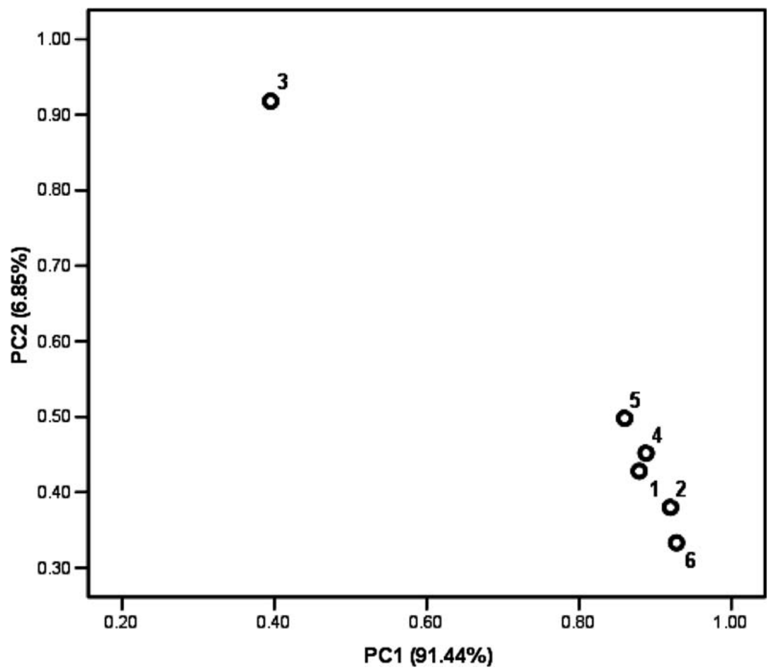

Fig. 4. Principal component analysis (PCA) of chemical constituents of essential oils of the six populations. The first two principal components (PC1 and PC2) contain about 98.29% information. 1, Chifeng-Wusan; 2, Chifeng-Sanyanjin; 3, Zhengxiangbaiqi; 4, Hohhot; 5, Baotou; 6, Ertokeqi.

acids (Table 1), contained high concentrations of phytol (15.73%), γ-eudesmol (7.77%), and eudesm-7(11)-en-4-ol (6.13%); while the others rich in monoterpene showed high contents of α-terpineol (36.56–52.23%) and *p*-vinylanisole (1.55–13.74%), and low contents of phytol (1.24–6.87%), γ-eudesmol (0–0.93%), and eudesm-7(11)-en-4-ol (0.41–0.57%). It can be concluded that two main chemotypes of essential oils of *E. sinica* may be identified by the characteristic concentrations of the five major compounds.

Although the two populations from Chifeng-Wusan and Chifeng-Sanyanjin are grouped into the same chemotype due to the fact that both oils contain about 50% α-terpineol, it should be noted that there are many variations between them. For example, the oils from Chifeng-Wusan contain 8.99% tetrapyrazine, 5.44% 3-methyl-2-buten-1-ol, and 1.55% *p*-vinylanisole, while in the oils from Chifeng-Sanyanjin 0.71% tetrapyrazine, 0.04% 3-methyl-2-buten-1-ol, and 11.64% *p*-vinylanisole are the major compounds. In general, our study suggests that the genetic and environmental factors should be taken into account in order to obtain stable quality of *E. sinica* for extracting essential oils.

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