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Variations in Essential Oil Content and Compositions of Eight *Zanthoxylum bungeanum* Species

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ABSTRACT

Zanthoxylum bungeanum has been much investigated while there have been virtually no comparison of its chemical compositions. Eight *Zanthoxylum bungeanum* species: Chenjiawuci (CW), Dangcunwuci (DW), Fengjiao (FJ), Hanchengdahongpao (HD), Hanchengwuci (HW), Qin'anyihao (QY), Shizitou (SZ) and Xinongwuci (XW) were collected in China. The essential oils from the eight species were obtained by simultaneous distillation extraction and evaluated by gas chromatography-mass spectrometry (GC–MS). Hierarchical clustering analysis was conducted to evaluate the relationship among the eight species based on contents of chemical constituents in essential oils. Nighty-eight compounds were identified from all the species and their quantities are different quantity among the eight species. Terpinen-4-ol and Terpinyl acetate were the main components with contents DW (21.17% and 15.62%), HD (18.67% and 14.43%), HW (31.44% and 9.43%), QY (21.95% and 15.74%), SZ (25.83% and 10.18%), XW (13.05% and 19.36%). On the other hand, Terpinen-4-ol (25.20%) and Cymene (10.08%) were the main components for CW while Terpinen-4-ol (12.08%) and Linalool (28.71%) were the main components for CW. CW and HW exhibited high similarity. The study facilitates linking the oil compounds with the pharmacological activities of essential oils extracted from *Z. bungeanum*. These results can provide valuable reference information for resource assessment and the rational utilization of *Z. bungeanum* resources.

Keywords: GC–MS; Essential oil; *Zanthoxylum bungeanum*; Chemical components; Variations

Abbreviations

ZBEO: *Z. bungeanum* Essential Oil; CW: Chenjiawuci; DW: Dangcunwuci; FJ: Fengjiao; HD: Hanchengdahongpao; HW: Hanchengwuci; QY: Qin'anyihao; SZ: Shizitou; XW: Xinongwuci; GC-MS: Gas Chromatography-Mass Spectrometry

Introduction

Zanthoxylum bungeanum is wildly distributed in most parts of China and some Southeast Asian countries. The fruits of this species are called 'da hong pao' (big red robe). The pericarps of the fruits of *Z. bungeanum* have been utilized as a pungent food stuff and traditional Chinese medicine for treatment of vomiting, toothache, stomach ache, abdominal pain, eczema and diarrhea [1-6]. Essential oils are volatile, natural, complex compounds characterized by a strong odour and are formed by aromatic plants as secondary metabolites. Essential oils have been widely used for bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic applications, especially nowadays in pharmaceutical, sanitary, cosmetic, agricultural and food industries [7]. Previous *Z. bungeanum* essential oil (ZBEO), which is from the pericarps of *Z. bungeanum*, researching has focused on the essential oil extraction, chemical composition and its biological activities such as antioxidant [8-10], antitumor [11-13], anti-inflammatory [14], antimicrobial and insecticidal [15,16].

To the best of our knowledge, few information on comparsion of the chemical composition of ZBEO is available. Thus, this study investigated the chemical composition of essential oils from different *Z. bungeanum* species and illustrated the chemical polymorphism among different *Z. bungeanum* species based on the characteristics of essential oils. The study facilitates linking the oil compounds with the pharmacological activities of essential oils extracted from *Z. bungeanum*. These results can provide valuable reference information for resource assessment and the rational utilization of *Z. bungeanum* resources

Materials and Methods

Plant materials

Eight *Z. bungeanum* species: Chenjiawuci (CW), Dangcunwuci (DW), Fengjiao (FJ), Hanchengdahongpao (HD), Hanchengwuci (HW), Qin'anyihao (QY), Shizitou (SZ) and Xinongwuci (XW) were collected from different regions of China (Table 1). CW, DW, HD, HW, QY, SZ and XW were transplanted to Northwest A&F University FENGXIAN experiment and demonstration station of Chinese prickly ash (Shaanxi Province, China) for at least three years and FJ was cultivated in the station. All the species were harvested as mature products in August 2014. Every sample contained pericarps from multiple trees of the corresponding species. Identification of these eight species was confirmed by Prof. An-zhi Wei from College of Forestry, Northwest A&F University, China. A voucher specimen was deposited in the college of forestry, Northwest A&F University (Yangling, China). All the pericarps of the eight species were stored at 4°C for use.

Essential oil extraction

The air-dried pericarps of eight *Z. bungeanum* species were powdered using a laboratory mill and filtered to 50 meshes. One hundred g of each sample powder was soaked in 1L of deionized water. For hydrodistillation extraction, the sample mixtures were extracted under atmospheric distillation for 3 h in Clevenger type apparatus to produce essential oil. The obtained essential oil was dried over anhydrous sodium sulphate and then extracted with a 0.2μm pore size filtration paper. It was kept in a sealed opaque vial at -20°C for subsequent experiments. The essential oils yield of every species was conducted five times.

Gas Chromatography-Mass Spectrometry analysis

The chemical components of essential oil in this study were analyzed by a GC-MS system (Thermo SCIENTIFIC), equipped with an HP-5MS capillary column (30 m × 0.25 mm; 0.25μm film thickness). The setting of temperature programming was as follows: 50°C (2min), 50-150°C at 3°C/min, 150-240°C at 8°C/min, 240°C (2 min). For the injection (split injector), 5μl sample was diluted in 620μl chloroform and then 1μl of the solution was injected. The injector temperature, MS transfer line temperature and ion source temperatures are maintained at 250, 280 and 250°C, respectively. The split ratio was 1:100, scan time was 3.5 min and helium (99.99%) was used as carrier gas at a flow rate of 1ml/min. The mass spectrometer was operated in EI mode at 70 eV in m/e range 45-500 amu.

Table 1: *Zanthoxylum bungeanum* species collected from different regions of China.

sample name	Sources	Growth years
Chenjiawuci (CW)	Han Cheng, Shaanxi province, China	3
Dangcunwuci (DW)	Han Cheng, Shaanxi province, China	3
Fengjiao (FJ)	Feng Xian, Shaanxi province, China	4
Hanchengdahongpao (HD)	Han Cheng, Shaanxi province, China	3
Hanchengwuci (HW)	Han Cheng, Shaanxi province, China	3
Qin'anyihao (QY)	Qin An, Gansu province, China	4
Shizitou (SZ)	Han Cheng, Shaanxi province, China	3
Xinongwuci (XW)	She Xian, Hebei province, China	4

Identification of the essential oil components

The identification of the components was performed by matching their recorded mass spectra with the standard massspectra from the National Institute of Standards and Technology (NIST08. LIB) library data provided by the software of the GC–MS system, literature data and standards of the main components. The results were also confirmed by the comparison of the elution order of the compounds with their relative retention indices (determined with reference to a homologous series of normal alkanes) on the HP-5ms capillary column [17,18]. Quantitative analyses of each essential oil component (expressed as an area percentage) were carried out by a peak area normalization measurement, calculated as the mean value of injections from each sample.

Statistical analysis

Hierarchical cluster analysis (HCA) is a widely used classificatory technique in many areas of scientific knowledge. It is one of the most used classification methods in chemistry, being widely used in analytical chemistry, biochemistry, and multidisciplinary chemistry [19], which could discover the differences in species caused by multiple factors. By using HCA method on SPSS statistics 17.0 software (SPSS Inc., Chicago, IL, USA), and based on the content (%) of chemical constituents in essential oils, the relationship among the eight Z. bungeanum species were examined. For the same sample, and adopting different methods of system type is broadly in line with a result, the average clustering method is widely used so we sued the average method for clustering. The compositions above 1% were selected as the clustering variables.

Results and Discussion

The essential oils yield

Essential oils extracted from aromatic plants have a wide range of medicinal and industrial applications, such as antiseptic, preservatives, and antiphlogistic uses [20]. The total essential oil content of plants is generally very low and rarely exceeds 1% by mass. In the present study, the yield of essential oil strong varied for eight different *Zanthoxylum bungeanum* species. It fluctuated between 0.944 and 1.722% (v/w) and ranged from high to low: 1.722 ± 0.172% (CW), 1.314 ± 0.094% (FJ), 1.264 ± 0.093% (HD), 1.222 ± 0.050% (HW), 1.152 ± 0.093% (DW), 1.136 ± 0.062% (SZ), 0.951 ± 0.049% (QY), and 0.944 ± 0.045% (XW) (v/w). All the essential oils were colorless transparent and fragrant. There were many factors influenced the essential oil yields, such as species, different parts, harvesting time, developmental stages and environmental conditions [21,22]. Eight *Zanthoxylum bungeanum* species were cultivated in China under the four environmental conditions, including Han Cheng and Feng Xian, Shaanxi province, Qin An, Gansu province, She Xian, Hebei province (Table 1). Where are different from precipitation, temperature, solar radiation, and soil condition. Thus, the differences in essential oil yield and composition could mainly be attributed to the genotypes of *Zanthoxylum bungeanum* species and the environmental conditions.

Chemical composition of the essential oils

The detailed compositions of the essential oils identified by GC–MS displayed remarkable chemical polymorphism among the eight *Zanthoxylum bungeanum* species (Table 2). A total of 98 components were identified from all the samples including 17 components firstly reported in the essential oils of *Zanthoxylum bungeanum* species. Table 2 showed that 46, 52, 33, 41, 38, 49, 46, 62 compounds were identified from CW, DW, FJ, HD, HW, QY, SZ and XW, accounting for 100.0%, 99.12%, 97.92%, 99.55%, 99.50%, 99.43%, 99.44% and 98.81% of the total essential oils, respectively. According the previous study, a total of 35 and 39 essential compounds were analyzed in ZBEO while more compounds were identified in present study [23,24]. Discrepancies of species, growth condition, environmental conditions may be cause of the difference. Forty-three compounds which existed in more than half of the species (≥4), while the contents of every compound were greatly different. Thirteen compounds were shared among the essential oils of the eight *Z. bungeanum* species while the contents of every compound were also great different. The major shared components were Terpinen-4-ol (11.81-31.44%), Linalool (5.08-28.71%), Terpinyl acetate (5.45-19.36%), Terpineol (1.41-18.67%), Cymene (0.48-10.08%), Eucalyptol (1.5-9.43%), Linalyl acetate (0.59-9.38%) (Figure 1).

Our results revealed obvious differencesn the oil constituents of the eight *Z. bungeanum* species. The main components (above 3%) of the essential oils are as follows:

Table 2: Chemical compounds in the essential oils of eight *Zanthoxylum bungeanum* species.

RT(min)	RI [a]	Compound name	CW	DW	FJ	HD	HW	QY	SZ	XW
8.05	926	cis-4-Carene	— [b]	0.1	—	—	—	—	—	—
8.13	929	3-Carene	0.24	—	0.15	—	0.21	—	—	—
9.57	961	β-Phellandrene	—	0.15	—	—	—	0.24	—	0.25
9,70 [c]	970	Sabinene	1.01	0.16	—	—	0.65	—	0.36	—
10.25	983	L-β-Pinene	—	1.24	—	—	—	—	—	—
10.42	987	Myrcene	2.68	—	2.16	0.31	2.49	0.36	0.35	0.31
10.88	997	p-menthadiene	—	—	—	—	—	—	0.77	—
11.08	1000	3-Thujene	—	—	0.29	—	—	—	—	—
11.34	1008	4-Carene	—	1.64	—	—	—	—	—	—
11.56	1011	Terpinene	0.65	—	1.46	—	0.21	0.56	0.78	0.97
11.88	1078	Cymene	10.08	6.51	0.48	2.68	4.23	6.5	7.1	2.36
12.06 [d]	1021	Sylvestrene	—	3.17	—	5.2	—	—	2.75	—
12.08	1022	Limonene	7.12	—	11.93	—	8.1	4.88	—	2.29
12.14	1023	α-Phellandrene	1.47	0.5	0.71	0.2	2.66	1.8	—	0.7
12.19	1024	Eucalyptol	1.5	2.32	6.4	9.43	7.17	4.67	5.79	5.32
12.44	1029	Pinene	2.17	2.02	1.52	0.18	1.38	1.75	0.94	0.66
12.89 [d]	1037	α-Ocimene	0.25	0.26	0.68	—	—	0.52	—	0.15
13.40	1046	Terpinene	0.83	4.24	2.8	—	1.07	2.81	2.25	2.5
13.68	1055	cis-Terpineol	—	—	0.28	—	0.62	0.34	0.66	0.35
13.99	1056	Sabinene hydrate	0.19	0.56	—	0.27	—	—	—	—
14.62	1067	Terpinolene	0.62	1.04	0.52	—	0.38	0.72	0.56	0.61
15.33	1078	Linalool	8.06	8.53	28.71	6.8	8.13	8.12	8.62	5.08
16.05	1088	trans-Terpineol	1.02	0.93	0.3	—	1.16	—	0.52	0.47
16.42	1094	trans-1-methyl-4-(1-methylethyl)-2-Cyclohexen-1-ol	—	—	—	0.66	—	0.89	—	—
17.26	1105	cis-Terpineol	0.73	0.62	—	—	0.84	—	0.62	0.29
17.27	1106	cis-1-methyl-4-(1-methylethyl)-2-Cyclohexen-1-ol	—	—	—	0.37	—	0.34	—	—
17.77	1112	Citronellal	0.25	0.28	—	—	0.27	0.14	0.42	0.78
18.97	1128	Terpinen-4-ol	25.20	21.17	12.08	18.67	31.44	21.95	25.83	13.05
19.69	1136	Terpineol	5.95	2.86	5.17	8.97	7.14	6.84	7.16	1.41
20.39	1145	cis-Piperitol	0.35	0.17	0.18	0.46	0.42	0.18	—	0.09

20.49 [d]	1180	Z-11-Hexadecenoic acid	—	0.21	—	—	—	—	—	—
20.53 [d]	1146	Octyl acetate	—	—	—	—	—	0.15	—	—
21.01 d	1152	Isoascaridole	0.27	0.24	—	—	—	—	—	0.15
21.15	1153	cis-Geraniol	—	—	0.65	—	—	—	—	—
21.29 [d]	1155	Citronellol	—	—	—	—	—	—	—	0.27
22.19	1235	Linalyl acetate	1.05	1.77	9.38	4.45	1.1	0.83	0.73	0.59
22.21 [d]	1302	Linalyl butyrate	—	—	—	—	—	—	—	0.85
22.33	1166	Piperitone	3.41	2.36	2.42	4.07	1.04	0.85	1.23	0.86
22.56	1168	Geraniol	—	—	0.05	—	—	—	—	—
23.67	1275	Bornyl acetate	0.37	0.53	0.12	0.76	0.56	0.5	0.63	0.65
24.22	1185	Piperitol acetate	0.44	0.57	0.21	0.31	0.53	—	0.29	0.46
24.24	1290	4-Terpinenyl acetate	—	—	—	—	—	0.5	0.43	—
24.98	1308	Terpineol acetate	0.56	0.5	0.16	0.91	0.41	0.24	0.56	0.39
25.40 [d]	1319	Myrtenyl acetate	—	0.08	0.11	1.58	—	0.23	—	—
26.08 [d]	1335	exo-2-Hydroxycineole Acetate	0.26	0.19	0.1	0.56	0.17	0.22	0.34	0.24
26.22	1338	2-Hydroxycineole acetate	—	—	0.11	—	—	—	—	—
26.42	1343	Terpinyl acetate	8.85	15.62	5.45	14.43	9.43	15.74	10.18	19.36
26.65	1348	Citronellyl acetate	4.17	4.97	—	2.41	2.9	3.18	2.35	7.4
27.05	1357	Nerol acetate	0.43	0.36	0.86	0.82	—	0.23	0.2	0.66
27.35 [d]	1364	(R)-Lavandulyl acetate	0.27	—	—	—	0.18	—	—	—
27.89 [d]	1376	3,7-dimethyl-2,6-octadien-1-yl Acetate	—	0.39	—	—	—	—	—	—
27.96	1219	Geranyl acetate	0.27	0.32	1.59	3.68	0.44	2.33	0.6	2.28
28.25	1349	Elemene	1.05	1.71	—	1.1	—	1.14	1.8	2.45
29.46	1389	Caryophyllene	0.9	1.71	—	1.25	0.86	1.22	3.23	4.13
30.93	1435	Humulene	0.39	0.54	—	0.23	0.25	0.35	0.73	1.2
31.13 [d]	1441	α-Longipinene	—	—	—	—	—	—	—	0.15
31.34	1447	(-)-δ-Elemene	—	—	—	—	—	—	0.64	—
31.61	1455	γ-Cadinene	—	—	—	—	—	—	0.81	—
31.79	1461	γ-Muurolene	—	—	—	—	—	0.39	0.19	0.15
32.01	1467	α-Copaene	0.35	0.95	0.19	0.5	0.24	—	—	—
32.12	1470	D-Germacrene	—	—	—	—	—	1.08	0.92	1.69
32.32	1476	Eudesmene	—	—	—	—	—	—	—	0.09
32.38	1478	Selinene	—	—	—	—	—	—	—	0.12
32.44	1480	β-Copaene	—	—	—	—	—	—	—	0.09
32.55	1483	α-Acorenol	—	—	—	—	—	0.16	—	—
32.61	1485	Bicyclogermacrene	—	—	—	—	—	—	—	0.62
32.76	1489	Muurolene	0.29	0.27	—	0.22	—	0.22	0.46	0.71
32.95	1495	Germacrene A	—	—	—	—	—	—	—	1.08
33.06	1498	β-Acorenol	0.31	0.16	0.7	0.41	0.15	—	0.39	—
33.12	1499	α-Bulnesene	0.48	—	—	—	—	—	—	—
33.25	1503	D-Guaiene	—	—	—	—	—	0.33	—	—
33.32	1505	δ-Muurolene	0.3	0.55	—	0.39	—	—	—	—
33.38 [d]	1507	γ-Amorphene	—	—	—	—	—	—	—	1.27
33.54	1511	Cadinene	0.56	1.47	—	0.51	0.54	1.08	0.86	1.93
34.77	1545	α-Elemol	—	0.26	—	0.47	—	0.2	—	0.39
35.07	1553	(-)-Globulol	—	—	—	0.32	—	—	—	—

35.19	1557	Germacrene B	—	0.22	—	—	—	0.43	—	0.76
35.38	1562	trans-Nerolidol	—	—	—	0.99	—	0.69	—	—
35.62	1568	Spathulenol	0.22	0.37	—	—	—	—	0.57	—
35.79	1573	(-)-Spathulenol	—	—	—	0.67	—	—	—	—
35.92	1576	Caryophyllene oxide	0.84	0.69	—	1.07	0.29	0.33	1.17	1.53
36.70	1597	Globulol	—	—	—	—	—	—	—	0.16
36.85 [d]	1601	Humulene epoxide	—	—	—	—	—	—	—	0.22
37.38	1614	Cubenol	—	0.14	—	—	—	—	0.16	0.29
37.52	1618	α-Guaiene	—	—	—	0.2	—	0.13	—	0.31
37.78	1616	Cadinol acetate	—	—	—	—	—	—	—	1.23
37.79	1624	Cadinol	0.62	0.64	—	0.51	0.27	0.5	0.83	—
37.83 [d]	1625	epi-α-Muurolol	—	0.72	—	0.59	0.41	—	—	—
37.88	1627	Muurolol	0.71	—	—	—	—	0.62	0.86	1.4
37.95	1628	Torreyol	—	0.25	—	—	—	0.14	0.23	0.39
38.12	1633	β-Cadinol	2.26	1.89	—	1.94	1.16	—	2.3	3.46
38.25	1636	α-Cadinol	—	—	—	—	—	1.81	—	—
38.74	1662	cis-Vaccenic acid	—	—	—	—	—	—	—	0.1
39.37	1663	Ledene oxide	—	—	—	—	—	—	—	0.16
41.71 [d]	1825	trans-Geranylgeraniol	—	—	—	—	—	—	—	0.26
41.95 [d]	1842	Cembrene	—	—	—	—	—	—	0.27	—
43.43	1945	Camphorene	—	—	—	—	—	—	—	0.45
44.45 [d]	2014	Manoyloxide	—	—	—	—	—	—	—	0.22
Total			100	99.12	97.92	99.55	99.50	99.43	99.44	98.81

[a]Retention indices on HP-5MS capillary column.
[b]Not detected.
[c]The content of the compounds above 1% used the bold font.
[d]Firstly reported in the essential oils of *Zanthoxylum bunge*anum species.

Terpinen-4-ol (25.20%), Cymene (10.08%), Terpinyl acetate (8.85%), Linalool (8.06%), Limonene (7.12%), Terpieonl (5.95%), Citronellyl acetate(4.17%), Piperitone (3.41%) in CW; Terpinen-4-ol (21.17%), Terpinyl acetate (15.62%), Linalool (8.53%), Cymene (6.51%), Citronellyl acetate (4.97%), Terpinene (4.24%), Sylvestrene (3.17%) in DW; Terpinen-4-ol (18.67%), Terpinyl actate (14.43%), Eucalyptol (9.43%), Terpieonl (8.97%), Linalool (6.80%), Sylvestrene (5.20%), Linalyl acetate (4.45%), Piperitone (4.07%), Geranyl acetate (3.68%) in HD; Terpinen-4-ol (31.44%), Terpinyl acetate (9.43%), Linalool (8.13%), Limonene (8.10%), Eucalyptol (7.17%), Terpieonl (7.14%), Cymene (4.23%) in HW; Terpinen-4-ol (25.83%), Terpinyl acetate (10.18%), Linalool (8.62%), Terpieonl (7.14%), Cymene (7.10%), Eucalyptol (5.79%), Caryophyllene (3.23%) in SZ; Linalool (28.71%), Terpinen-4-ol (12.08%), Limonene (11.93%), Linalyl acetate (9.38%), Eucalyptol (6.40%), Terpinyl acetate (5.45%), Terpieonl (5.17%) in FJ; Terpinen-4-ol (21.95%), Terpinyl acetate (15.74%), Linalool (8.12%), Terpieonl (6.84%), Cymene (6.50%), Limonene (4.88%), Eucalyptol (4.67%), Citronellyl acetate (3.18%) in QY; Terpinyl acetate (19.36%), Terpinen-4-ol (13.05%), Citronellyl acetate (7.40%), Eucalyptol (5.32%), Linalool (5.08%), Caryophyllene (4.13%), β-Cadinol (3.46%) in XW.

Above these results showed some differences in the major composition with previous reports: D-Limonene (22.19%), β-yrcene (9.66%), trans-β-Ocimene (9.58%),Terpinen-4-ol (8.96%), γ-Terpinene (4.45%), cis-β-Ocimene (4.28%), Eucalyptol (3.92%), β-Phellandrene (3.35%), Terpinyl actate (3.28%) and Linalool (3.15%) were reported by Li, et al. [23]; Terpinen-4-ol (18.42%), Eucalyptol (15.49%), limonene (7.47%), α-terpineol (5.79%), γ-terpinene (5.62%), terpinyl acetate (4.62%), and linalool (4.55%) were reported by Lan, et al. [6]. From Table 2, Terpinen-4-ol and Terpinyl acetate were found in essential oils of all *Z. bungeanum* species. They were the main components with contents in DW (21.17% and 15.62%), HD (18.67% and 14.43%), HW (31.44% and 9.43%), QY(21.95% and 15.74%), SZ (25.83% and 10.18%), XW (13.05% and 19.36%), respectively. On the other hand, Terpinen-4-ol (25.20%) and Cymene (10.08%) were the main components of CW essential oil while Terpinen-4-ol (12.08%) and Linalool (28.71%) were the main components for FJ. The contents of other compounds were all less than 10%. They were different from the previous reports [6,24,25]. These changes in the essential oil composition may arise from environmental conditions and genetic difference and also show the essential oils have abundant diversity.

Generally, the major components determine the biological properties of the essential oil. For the essential oils of the eight *Z. bungeanum* species, Terpinen-4-ol was the main compound. So the biological activities of their essential oils were directly related with Terpinen-4-ol, which was in accordance with previous work that reported it as an effective antiinflammatory, antioxidant and antimicrobial [26-31], depressant and anticonvulsant [32], and insecticidal [33-38]. It has been reported that Terpinen-4-ol could enhance the transdermal permeation of the polar and non-polar drugs [39], Eucalyptol, terpineol [40], and limonene could also do [41], indicating these major oil contributors play a crucial role in the enhancement activity of ZBEO as a transdermal penetration enhancer [6]. Besides, there were many studies about the apoptosis induce activities of Terpinen-4-ol in human cells [27-30]. These conformed to the result in our previous study that ZBEO has an observable effect on HaCaT cell apoptosis [24]. Based on the high content of Terpinen-4-ol. ZBEO are supposed to possess the aforementioned biological activities of Terpinen-4-ol.

Essential oils diversity

To evaluate the diversity of the ZBEO in the eight *Z. bungeanum* species the identified compounds were classified by their chemical structure (Table 3). Nine types of compounds were found in DW and XW, more than the compounds found in other six species. FJ has the least types of compounds. Alcohol (59.32%-33.07%), lipid (33.72%-15.31%) and olefin (25.86%-10.56%) are the most abundant three compositions of all eight species while other compositions are all below 10%. This phenomenon was caused by the highest content of Terpinen-4-ol and Terpinyl acetate, which were classified as alcohol and olefin, respectively. The result was similar to the previous study, in which the main compounds were oxygenated (41.65%) and non-oxygenated hydrocarbons (52.65%) [15]. Besides, the acid only existed in DW and XW while the cycloparaffin only existed in CW, DW and XW.

Relationship of the eight Zanthoxylum bungeanum species

The interspecies relationship of the eight *Z. bungeanum* species based on the 35 high content compositions (more than 1%, the bold font in Table 2) of their essential oils was calculated by hierarchical cluster analysis (HCA) in this study. HCA is a method used to measure the similarities among species by examining interpoint distances, representing all possible sample pairs in high dimensional space [42]. In the analysis, the chosen 35 high content compositions of eight species formed a matrix of 35 × 8. Analysis of the data was performed using the Statistics software SPSS. The results showed that the XW has a single cluster from other seven species while among this seven species, HD, FJ and WD have particular separate clusters. Based on the distance of the cluster, CW and HW have the closest relationship. QY and SZ also exhibited significant similarity but less than the CW and HW (Figure 2).

Conclusion

In this work, a direct comparison of chemical compositions in essential oils of eight *Z. bungeanum* species and their interspecies relationship were researched for the first time. Terpinen-4-ol and Terpinyl acetate were the major compounds of essential oil in DW, HD, HW, QY, SZ and XW. Terpinen-4-ol and Cymene were the main components of essential oil in CW while Terpinen-4-ol and Linalool were the main components of essential oil in FJ. CW and HW exhibited the highest similarity based on the high content compositions in their essential oils. The present study facilitates linking the oil compounds with the pharmacological activities of essential oils extracted from *Z. bungeanum*. These results can provide valuable reference information for resource assessment and the rational utilization of *Z. bungeanum* resources.

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Author Contributions

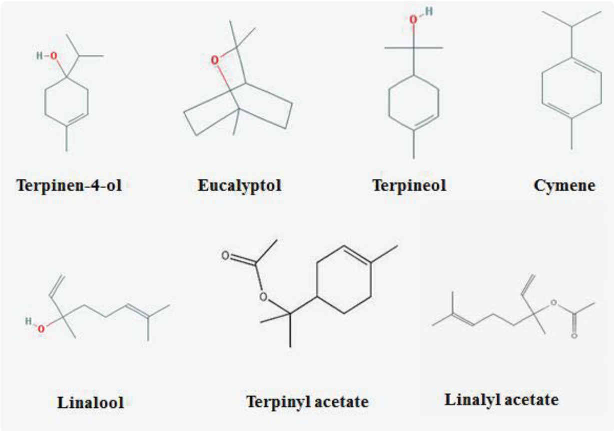
Keyou Li conceived and designed the project; Yonghong Liu collected material; Zhe Li and Wang-wang Jia hydrodistilled the essential oil; Hailan Zhu analyzed the data; Hailan Zhu and Keyou Li wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Table 3: The diversity essential oils of eight *Zanthoxylum bungeanum* species.

Compound type	Total number of compounds	CW (%)	DW (%)	FJ (%)	HD (%)	HW (%)	QY (%)	SZ (%)	XW (%)
Alcohol (number)	29	46.93(12)	41.03(15)	54.48(10)	51.26(15)	58.91(12)	47.45(15)	54.54(14)	32.68(16)
Olefin (number)	43	21.36(18)	21.94(19)	22.41911)	10.29(12)	19.04(13)	20.01(19)	18.40(17)	25.86(27)
Lipid (number)	**17**	16.67(10)	25.30(110	18.09910)	29.91(10)	15.72(9)	24.15(11)	16.31(10)	34.11(11)
Substituted benzene(number)	1	10.08 (1)	6.51 (1)	0.48 (1)	2.68 (1)	4.23 (1)	6.50 (1)	7.10 (1)	2.36 (1)
Ketone (number)	1	3.41 (1)	2.36 (1)	2.42 (1)	4.07 (1)	1.04 (1)	0.85 (1)	1.23 (1)	0.86 (1)
Olefin oxide (number)	3	0.84 (1)	0.69 (10)	—	1.07 (1)	0.29 (1)	0.33 (1)	1.17 (1)	1.91 (3)
Cycloparaffin(number)	1	0.27 (1)	0.24 (1)	—	—	—	—	—	0.15 (1)
Aldehyde (number)	1	0.25 (1)	0.28 (1)	—	—	0.27 (1)	0.14 (1)	0.42 (1)	0.78 (1)
Acid (number)	2	—	0.21 (1)	—	—	—	—	—	0.10 (1)



Figures 1: Chemical structures of some major compounds of essential oils from eight Z. bungeanum species.

Figures 2: Relationship of the eight Zanthoxylu.

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