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Characteristic differences in essential oil composition of six *Zanthoxylum bungeanum* Maxim. (Rutaceae) cultivars and their biological significance*#

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Great variations have been found in composition and content of the essential oil of *Zanthoxylum bungeanum* Maxim. (Rutaceae), resulting from various factors such as harvest time, drying and extraction methods (Huang *et al*., 2006; Shao *et al*., 2013), solvent and herbal parts used (Zhang, 1996; Cao and Zhang, 2010; Wang *et al*., 2011). However, in terms of artificial introduction and cultivation, there is little research on the chemical composition of essential oil extracted from *Z. bungeanum* Maxim. cultivars, which have been introduced from different origins. In this study, the composition and content of essential oil from six cultivars (I–VI) have been investigated. They were introduced and cultivated for 11 years in the same cultivation conditions. Cultivars were as followings: Qin'an (I) cultivar originally introduced from Qin'an City in Gansu Province; Dahongpao A (II) from She County in Hebei Province; Dahongpao B (III) from Fuping County; Dahongpao C (IV) from Tongchuan City; Meifengjiao (V) from Feng County; and, Shizitou (VI) from Hancheng City, in Shaanxi Province, China. This research is expected to provide a theoretical basis for further introduction, cultivation, and commercial development of *Z. bungeanum* Maxim.

Seventy-one components, representing 99.07%–99.78% of the essential oil, were identified and quantified in the essential oils extracted from all six *Z. bungeanum* cultivars I–VI (Table S1). There were characteristic differences in total icon chromatograms (TICs) of volatile components of the six *Z. bungeanum* Maxim. cultivars (Fig. 1). Sylvestrene (at 11.55 min) was 26.54%, 23.51%, 17.75%, and 24.66% for the Dahongpao A–C (II–IV) and Shizitou (IV), respectively, but it was not detected in others. The characteristic peak of (*Z*)-4-thujanol (retention index (RI)= 1041) for Dahongpao B (III) occurred at 13.37 min (area, 2.69%), but there was little (≤0.11%) of this compound in others. For Meifengjiao (V), the linalyl butanoate (RI=1471) content was 20.80% at 22.01 min, but this component was less than 1.00% in others.

It was noteworthy that the Qin'an (I) cultivar was distinguished by the abundance of limonene, up to 60.53%, followed by β-myrcene (15.34%). However, this value was reasonable when it was compared with previous findings of high levels of estragole (69.52%) in *Zanthoxylum schinifolium* fruits (Wang *et al*., 2011).

Forty-one main constituents which exceeded 0.10% and 29 shared compounds were presented in six cultivars (Table 1). The constituents of volatile oils varied significantly among the cultivars, as did the contents of each component. This might be the cause of characteristic differences in the aroma of the oils. The total content of β-myrcene, limonene,

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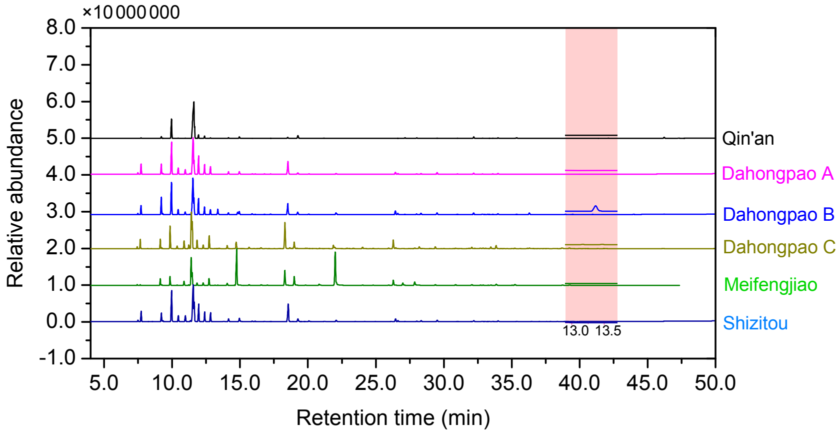

Fig. 1 Stacking chart of total icon chromatograms (TICs) of volatile components in the pericarp of six cultivars

sylvestrene, linalool, ocimene, terpinen-4-ol, and linalyl butanoate exceeded 50%. These compounds were indicators of the quality and were responsible for the differences (especially the aroma) among *Z. bungeanum* varieties. Some especially volatile compounds such as germacrene D-4-ol (0.98%) were only present in the essential oil from Dahongpao B (III).

The variations among cultivars were analyzed by cluster analysis (Fig. S1). At a distance of 10, six cultivars could be classified into three groups. Dahongpao A (II), B (III), C (IV), and Shizitou (VI) could be assigned to the same group, because the total contents of six main compounds, β-myrcene, (*E*)/(*Z*)-β-ocimene, sylvestrene, eucalyptol, and terpinen-4-ol, were up to 53.46%–70.42%; The cultivar Qin'an (I), was an independent group because of the unusual presence of α-limonene (60.53%); Meifengjiao (V) also formed an independent group, because of being different from others due to the presence of different main constituents, β-linalool (21.17%) and linalyl butanoate (20.80%), while others had less than 3%. α-Limonene, linalool, linalyl butanoate, and terpinen-4-ol were the main components of Meifengjiao (V), up to 63.73%.

After 11 years' cultivation, both chemical compositions and contents of six *Z. bungeanum* Maxim. cultivars were obviously different from those reported in previous studies. In this research, for cultivar Shizitou (VI) from Hancheng, sylvestrene (up to 24.66%) and β-myrcene (14.91%) were the major constituents while limonene (14.82%), eucalyptol (15.64%), or sabinene (7.93%) were in previous reports (Huang *et al*., 2006; Shao *et al*., 2013). In addition, for Meifengjiao (V), Shao *et al*. (2013) reported that β-phellandrene (42.29%) was the principal compound while β-linalool (21.17%) and linalyl butanoate (20.80%) took those positions in this study.

Dahongpao A–B (II, III) and Shizitou (VI), which were rich in D-sylvestrene, would have higher defense levels than Meifengjiao (V) because D-sylvestrene was a wound signal constituent (Fan *et al*., 2015). This has been also shown by previous plant physiological research (Liu *et al*., 2010).

We believe that Dahongpao B (III) and C (IV) also have favorable antitumor potential mainly due to elemene, an antineoplastic agent (Yang *et al*., 1997), up to 0.74% for III and 0.95% for IV (Gong *et al*., 2009; Li Z.D. *et al*., 2013; Li K.Y. *et al*., 2016). In addition, Dahongpao C (IV) may have a better antifungal activity because of richness in α-pinene and terpinen-4-ol which are both antifungal active components (Gong *et al*., 2009; Qian, 2011; Li and Xue, 2014); Qin'an (I) maybe have excellent antibacterial activity because it contains plenty of limonene which has shown clear antibacterial activity (Zhu *et al*., 2011); Meifengjiao (V) may exhibit favorable insecticidal activity because of high levels of linalool which was the major active ingredient (Zhang *et al*., 2016).

In summary, characteristic differences have been found in composition and content of essential oil from the pericarp of six *Z. bungeanum* Maxim. cultivars. Furthermore, there are obvious variations between species planted in original places and cultivars. Different cultivars have different favorable potential because of different characteristic components. It is expected that this research can provide a theoretical basis for further introduction, cultivation, and commercial development of *Z. bungeanum* Maxim.

Table 1 Main chemical composition of essential oils from the pericarp of six *Z. bungeanum* cultivars

RI[a]	Compound	RA[b] (%)					
		I[c]	II	III	IV	V	VI
902	α-Thujene	0.06±0.01	0.58±0.01	0.43±0.01	0.62±0.02	0.25±0.01	0.56±0.01
919	4-Carene	0.57±0.02	1.29±0.02	0.88±0.01	1.44±0.02	0.98±0.01	1.35±0.01
943	β-Pinene	0.25±0.01	0.37±0.01	0.40±0.01	0.34±0.02	0.19±0.01	0.38±0.01
948	α-Pinene	0.34±0.01	4.19±0.01	3.34±0.01	3.15±0.02	0.15±0.01	3.87±0.01
958	β-Myrcene	**15.34±0.02**[d]	**17.50±0.02**	**15.71±0.01**	**8.33±0.02**	3.59±0.02	**14.91±0.02**
964	β-Phellandrene	1.22±0.02	4.43±0.01	**7.07±0.02**	3.39±0.01	2.60±0.01	3.42±0.02
969	α-Phellandrene	0.30±0.01	2.87±0.01	2.11±0.01	1.05±0.01	0.07±0.00	2.54±0.01
976	(*E*)-β-Ocimene	2.49±0.01	**9.04±0.02**	**7.05±0.01**	3.39±0.01	0.99±0.01	**7.75±0.01**
986	(*Z*)-β-Ocimene	1.66±0.01	4.39±0.01	3.27±0.02	1.46±0.01	1.07±0.01	4.03±0.01
998	α-Terpinene	0.26±0.01	2.17±0.01	1.15±0.02	3.06±0.01	0.16±0.01	2.55±0.01
1014	Sylvestrene		**26.54±0.02**	**23.51±0.03**	**17.75±0.02**		**24.66±0.03**
1018	α-Limonene	**60.53±0.05**				**13.67±0.02**	
1037	γ-Terpinene	0.44±0.01	3.54±0.01	2.10±0.02	**5.30±0.02**	3.04±0.01	3.89±0.01
1041	(*Z*)-4-Thujanol	0.03±0.00	0.02±0.00	2.68±0.02	0.11±0.01	0.09±0.00	
1042	Cymene		0.53±0.01	0.20±0.02	1.57±0.01	1.72±0.02	0.50±0.01
1059	Eucalyptol		4.92±0.01	**5.64±0.02**	**9.17±0.01**	4.86±0.01	**6.63±0.01**
1082	β-Linalool	1.55±0.02	1.57±0.01	1.73±0.02	2.72±0.01	**21.17±0.02**	1.82±0.01
1109	(*E*)-*p*-Menth-2-en-1-ol	0.10±0.00	0.17±0.01	1.31±0.02	0.67±0.01	0.27±0.01	0.24±0.01
1126	(*Z*)-*p*-Menth-2-en-1-ol	0.04±0.01	0.26±0.01	0.14±0.01	0.05±0.00	0.24±0.01	0.15±0.01
1137	Terpinen-4-ol	1.08±0.01	**8.03±0.02**	**6.09±0.02**	**13.36±0.03**	**8.09±0.02**	**10.62±0.01**
1143	α-Terpineol	3.07±0.01	0.85±0.01	1.46±0.02	3.23±0.01	4.88±0.02	1.64±0.01
1158	Piperitone	0.13±0.01	0.47±0.01	1.11±0.01	1.76±0.01	0.13±0.01	0.83±0.01
1228	Geraniol	0.47±0.01		0.19±0.01		0.62±0.01	0.04±0.00
1302	Citronellyl acetate	0.21±0.02	0.35±0.01	0.68±0.01	0.50±0.01		0.51±0.01
1333	α-Terpineol acetate	0.19±0.01	1.09±0.01	2.02±0.01	4.30±0.01	2.87±0.01	1.50±0.01
1352	Nerol acetate	0.67±0.02	0.06±0.00	0.14±0.01	0.14±0.01	1.39±0.01	0.08±0.00
1370	Geraniol acetate	0.59±0.02	0.22±0.01	0.39±0.01	0.32±0.01	2.39±0.01	0.26±0.01
1398	Elemene	0.09±0.00	0.30±0.00	0.74±0.02	0.95±0.02	0.17±0.02	0.49±0.01
1431	Elixene	0.41±0.01	0.18±0.01	0.32±0.01		0.08±0.01	0.18±0.01
1435	γ-Cadinene	0.29±0.01	0.18±0.00	0.20±0.01	0.77±0.01	0.14±0.02	0.16±0.01
1440	α-Muurolene	0.14±0.01	0.09±0.01	0.11±0.01	0.37±0.01	0.10±0.01	0.09±0.00
1458	α-Farnesene	0.20±0.01	0.09±0.00	0.11±0.01		0.05±0.00	0.08±0.00
1469	δ-Cadinene	0.69±0.01	0.46±0.01	0.51±0.01	1.69±0.01	0.54±0.02	0.42±0.02
1471	Linalyl butanoate				0.54±0.01	**20.80±0.03**	
1494	Caryophyllene	0.36±0.01	0.96±0.01	0.98±0.01	0.97±0.01	0.31±0.02	0.73±0.01
1515	Germacrene D	1.46±0.02	0.58±0.01	0.92±0.02	0.70±0.01	0.34±0.01	0.54±0.01
1579	α-Caryophyllene	0.29±0.01	0.15±0.01	0.22±0.01	0.23±0.01	0.29±0.01	0.13±0.01
1580	τ-Cadinol	0.19±0.01	0.06±0.00	0.15±0.01	0.50±0.00	0.15±0.01	0.07±0.00
1593	α-Cadinol	0.27±0.01	0.05±0.00	0.15±0.01	0.37±0.00	0.22±0.01	0.07±0.00
1603	Germacrene B	0.70±0.01	0.06±0.00	0.20±0.01		0.43±0.01	0.11±0.00
1660	Germacrene D-4-ol			0.98±0.01			
	Total content	96.68	98.62	96.39	94.28	99.11	97.80

[a] RI indicates the retention index calculated against C_8–C_{30} *n*-alkanes on the Rxi-5MS capillary column. [b] RA, relative area, is the percentage of peak area relative to the total peak and each value represents the mean±standard deviation (SD) with n=3. [c] I–VI mean different cultivars: Qin'an (I) cultivar originally introduced from Qin'an City in Gansu Province; Dahongpao A (II) from She County in Hebei Province; Dahongpao B (III) from Fuping County; Dahongpao C (IV) from Tongchuan City; Meifengjiao (V) from Feng County; and, Shizitou (VI) from Hancheng City, in Shaanxi Province, China. [d] Values in bold indicate component areas >5.0%

Materials and methods

Detailed information on materials and methods is available from supplementary information Data S1.

Contributors

Prof. Shu-ming LIU and Prof. Bing-yin SUN provided the research samples and funding support; Prof. Shu-ming LIU and Jun-ru Wang designed the experiments; Shi-jun WANG, Si-yao SONG, and Yong ZOU performed the experiments; Shi-jun Wang analyzed the data and wrote the paper. All of the authors have approved the final version of the manuscript.

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Compliance with ethics guidelines

Shu-ming LIU, Shi-jun WANG, Si-yao SONG, Yong ZOU, Jun-ru WANG, and Bing-yin SUN declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 Volatile components from all six *Z. bungeanum* cultivars I–VI

Fig. S1 Cluster analysis results for six *Z. bungeanum* Maxim. cultivars

Data S1 Materials and methods

中文概要

题　目：六个花椒栽培种挥发油组分的特征性差异及生物学意义

概　要：从花椒人工引种栽培出发，通过气相色谱法-质谱法联用法（GC-MS）分析了6个不同花椒栽培种的挥发油组分，据此进行了聚类分析，研究结果如下。（1）占挥发油99.07%~99.78%的71种化合物得以鉴定，其中29种为6个栽培种共有。（2）聚类分析将6个品种分为3类：Dahongpao A~C（II~IV）与Shizitou（VI）聚为一类，因为有6个主化合物相同，为β-月桂烯、(*E*)/(*Z*)-β-罗勒烯、枞油烯、桉树脑和4-羟基松油醇，占挥发油的53.46%~70.42%；Qin'an（I）单独一类，因为其α-柠檬烯占挥发油的60.53%；Meifengjiao（V）也单独成类，其主成分和其他品种均不同，含21.17%β-芳樟醇和20.80%乙酸芳樟酯，而其他品种的这些成分却低于3%。（3）Dahongpao B/C、Qin'an和Meifengjiao分别具有良好的抗肿瘤、抗真菌、抗细菌及杀虫潜力。

关键词：花椒栽培种；挥发油；组成；特征性差异；气相色谱法-质谱法联用法（GC-MS）；聚类分析