Widely targeted metabolomic analysis reveals differences in volatile metabolites among four *Angelica* species

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Highlights

Eight hundred and ninety-nine volatile metabolites were identified in four *Angelica* species.

Medicinal plants differed in the accumulation of volatile compounds: *Angelica keiskei* is rich in bornyl acetate, while *Angelica sinensis* is rich in 7-hydroxycoumarin and Z-ligustilide.

*Angelica keiskei* exhibited high diversity and abundance of effective volatile compounds, and demonstrated its profound potential for industrial applications.
Abstract

Angelica L. has attracted global interest for its traditional medicinal uses and commercial values. However, few studies have focused on the metabolomic differences among the Angelica species. In this study, we analyzed volatile metabolites of four Angelica species (Angelica sinensis (Oliv.) Diels, Angelica biserrata (R.H. Shan & Yuan) C.Q. Yuan & R.H. Shan, Angelica dahurica (Hoffm.) Benth. & Hook.f. ex Franch. & Sav., Angelica keiskei Koidz.) by employing the widely targeted metabolomics based on gas chromatography–tandem mass spectrometry. A total of 899 volatile metabolites were identified and classified into sixteen different categories. On average, categorical abundances of volatile metabolites such as terpenoids, alcohol, ketone, and ester were higher in Angelica keiskei than those in the other three Angelica species. The metabolomic analysis indicated that 7-hydroxycoumarin and Z-ligustilide were accumulated at significantly higher levels in Angelica sinensis, whereas the opposite pattern was observed for bornyl acetate. In addition, we found a high correspondence between the dendrogram of metabolite contents and phylogenetic positions in the four species. This study provides a biochemical map for the exploitation, application and development of the Angelica species as medicinal plants or health-related dietary supplements.

Keywords

Angelica, volatile metabolites, Chinese traditional medicine, phylogeny
1. Introduction

*Angelica L.*, a genus in the family Apiaceae, is comprised of 90 species of herbs that are widespread in north-temperate regions, especially Eurasia (Feng et al., 2009; Sowndhararajan et al., 2017). Many plants in the genus have long been used in traditional Chinese medicine (TCM) (Sarker and Nahar, 2004), in particular, the dried roots of *Angelica* have been widely used for nourishing blood, regulating menstruation, and analgesic (Dong et al., 2022; Sowndhararajan et al., 2017). Various herbal preparations containing *Angelica* species are available over the counter, not only in China, but also in Europe and American countries (Hook, 2014; Wei et al., 2016). Besides its medicinal value, *Angelica* is also highly appreciated in various industrial applications such as the dietary supplements, perfumery, and cosmetics (Alkan Turkucar et al., 2021; Sowndhararajan et al., 2017; Zhang et al., 2012).

A previous study demonstrated that the pharmacological activity of aromatic and medicinal plants is attributed to its effective volatile components (Pandey et al., 2020). Plants in *Angelica* are extremely rich in secondary metabolites, including coumarins, flavonoids, terpenoids, as well as volatiles oils (VOs) (Sarker and Nahar, 2004; Sowndhararajan et al., 2017). Modern medical research has revealed that the VOs composition is mainly responsible for the medicinal properties of the genus *Angelica* (Kumar et al., 2022). VOs are complex mixture of low molecular weight volatile compounds that are isolated from the raw plant material by distillation (Sadgrove et al., 2022), which have been reported to treat serious health diseases, involving gynecological diseases, fever, and arthritis. (Perveen et al., 2020; Sowndhararajan et
There are a couple of good examples showing the proven effects of VOs in *Angelica* species. Phthalides of *A. sinensis* are one of the highly effective VOs to analgesic and sedative activities (Du et al., 2006; Wei et al., 2016). *Angelica biserrata* also contains active ingredients such as oxygenates, terpenoids, ketones and esters with analgesic and anti-inflammatory effects (Ma et al., 2019). However, most of current studies only focused on several targeted compounds in a single *Angelica* species. There have been no comprehensive and comparative studies examining the volatile metabolites of multiple *Angelica* species. It has posed a major obstacle to the application and exploitation of the medicinal plants in *Angelica* species.

With the development of metabolomics, high-throughput and high-resolution methods such as headspace solid phase micro-extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) have been widely used to identify metabolite profiles and detect differences in the biochemical compositions of aromatic and medicinal plants (Chen et al., 2021; Hua et al., 2019; Kumar et al., 2022). The four species *A. biserrata, A. dahurica, A. keiskei* and *A. sinensis* are the representative medicinal plants in *Angelica*, and it is noteworthy that roots of *A. sinensis* are one of the most widely prescribed medicine in China owing to its rich VOs (Wei et al., 2016).

In this study, volatile metabolites of four *Angelica* species were identified and quantified using widely targeted metabolomics. The aim was to reveal the differed accumulation of medicinally important metabolites among the four species. This study provides useful information for the chemical composition of *Angelica* plants.
and may help the identification of the biologically active substances responsible for the pharmacological activity of Angelica plants.

2. Materials and Methods

2.1. Plant samples

Four species in genus Angelica, including A. sinensis, A. dahurica, A. biserrate, and A. keiskei, were analyzed in this study. The A. sinensis plants were collected from Minxian County, Gansu Province, China. The A. dahurica, A. biserrate, and A. keiskei plants were collected from Shenzhen City, Guangdong Province, China. Roots of each species were sampled with three biological replicates. The collected roots were washed, naturally dried, frozen in liquid nitrogen, and then stored at -80°C for further analysis.

2.2. Solid phase microextraction (SPEM) extraction

The samples were ground into powder in liquid nitrogen. Powdered samples (1 g) were weighed and transferred immediately to a 20 mL head-space vial (Agilent, Palo Alto, CA, USA), containing NaCl saturated solution to inhibit potential enzyme reactions. The headspace vials were sealed using crimp-top caps. As for SPME analysis, each vial was placed in 60°C for 5 min, and then a 120 µm DVB/CWR/PDMS fiber (Agilent, Palo Alto, CA, USA) was exposed to the headspace of the sample for 15 min at 100°C. The quality control (mix) sample was prepared by mixing equal volumes of samples into a single tube to check the reproducibility of the Mass Spectrometry results.

2.3. GC-MS analysis
After the extraction procedure, the fiber was transferred to the injection port of the GC-MS system (Model 8890; Agilent, Palo Alto, CA, USA). The SPME fiber was desorbed and maintained in the injection port at 250°C for 5 min in the split-less mode. The identification and quantification of volatile metabolites was carried out using an Agilent Model 8890 GC and a 7000 D mass spectrometer (Agilent, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5% phenyl-polymethylsiloxane) capillary column. Helium was used as the carrier gas at a linear velocity of 1.2 mL min⁻¹. The injector temperature was kept at 250°C and the detector at 280°C. The oven temperature was programmed as followings: 40°C (3.5 min), increasing at 10°C min⁻¹ to 100°C, 7°C min⁻¹ to 180°C, 25°C min⁻¹ to 280°C and hold for 5 mins. Mass spectra was recorded in electron impact (EI) ionization mode at 70 eV. The quadrupole mass detector, ion source and transfer line temperatures were set, respectively, at 150, 230 and 280°C. For the identification and quantification of analytes, the MS was selected ion monitoring (SIM) mode.

2.4. Qualitative and quantitative analysis

After the mass spectrometry analysis, all raw data were analyzed with the software Qualitative Analysis Workflows B.08.00 (Agilent, Palo Alto, CA, USA). The qualitative analysis of primary and secondary mass spectrometry data was annotated based on the self-built database MWDB (Metware Biotechnology Co., Ltd. Wuhan, China) and the publicly available metabolite databases.

2.5 Statistical analysis
After the metabolite data was transformed with Hellinger transformation, principal component analysis (PCA) was performed using the function rda in the R package vegan v 2.6-2 (Oksanen et al., 2013). The pretreated data set of annotated metabolites were imported into the R package MetaboAnalystR v1.0.1 (Chong and Xia, 2018) to conduct orthogonal partial least squares-discriminant analysis (OPLS-DA) and extract the variable important in projection (VIP) value from the analysis results. In addition, paired sample student’s t test ($P$ value < 0.05) was used to determine the significance of the differences. Based on Bray-Curtis’s dissimilarity distances of the composition and abundance of volatile metabolites, which were calculated using the function vegdist built in vegan, hierarchical clustering was visualized with the R package factoextra v.1.0.7 (Lê et al., 2008).

The chloroplast sequence alignments of $A. \text{sinensis}$, $A. \text{dahurica}$, $A. \text{biserrate}$, and $A. \text{keiskei}$ were generated using MAFFT v7.475 (Katoh and Standley, 2013). Phylogenetic trees were constructed by maximum likelihood using IQ-TREE v 2.1.2 (Nguyen et al., 2015) with $\text{Hydrocotyle sibthorpioides}$ Lam. as an outgroup.

All identified metabolites were annotated with KEGG database (http://www.kegg.jp/kegg/compound/) and further subjected to KEGG enrichment analyses with the R package clusterProfiler v. 4.4.4 (Wu et al., 2021).

### 3. Results

#### 3.1. Metabolomics profiling of four Angelica species

To get insight into differences of volatile metabolites among four Angelica species, the root metabolomics data were generated. A total of 899 non-redundant
volatile metabolites were qualified and quantified based on GC-MS (Table S1). Among them, 673, 678, 730, and 793 volatile metabolites in *A. sinensis*, *A. dahurica*, *A. biserrate*, *A. keiskei*, respectively. In particular, 477 volatile metabolites were present in the roots of all four species (Fig. 1a).

PCA of the metabolome data, transformed with Hellinger transformation method, demonstrated metabolic divergence among the roots of the four *Angelica* species. Based on the PCA plot, where PC1 and PC2 explained 47.79% and 30.35% of the total variance, respectively, the samples were divided into four distinct groups corresponding to the four species. Of the four clusters, PC1 mainly differentiated *A. sinensis* from the other *Angelica* species, while PC2 primarily segregated *A. dahurica* from the other *Angelica* species.

The abundances of volatile metabolites were transformed by Z-score and then subjected to hierarchical clustering analysis (Fig. 1c). The results showed that the metabolites of the four *Angelica* species were evidently differentiated, and the three biological replicates were clustered together, which was consistent with the PCA plot. Taken together, these results suggested that volatile metabolites have diverged across the four *Angelica* species.

3.2. Specific characteristics of metabolites in four *Angelica* species

To explore the metabolite composition of the four species, 899 volatile metabolites were classified into 16 different categories, including terpenoids, ester, heterocyclic, aromatics and 12 others (Fig. 2). The terpenoids took up the highest proportion of all measured volatile metabolites in the four *Angelica* species, followed by heterocyclic
compounds, eater, and aromatics. Notably, *A. sinensis* contained a relatively lower proportion (41%) of terpenoids than other *Angelica* species, but exhibited a more balanced metabolite composition in volatile metabolites. In contrast, the amount of terpenoids accounted for more than half of the total volatile metabolites in *A. dahurica*, *A. biserrate*, and *A. keiskei*, especially in *A. dahurica*, its proportion reached up to 72.9%.

In addition, we compared the relative abundance of each metabolite category in the four species and found that the seven categories showed a significant difference among the four species, including alcohol, aromatics, aldehyde, ester, heterocyclic compounds, ketones and terpenoids (Fig. S1; Paired sample *t* test, the *p*-values were shown in the table S2). The relative content of terpenoids were significantly higher in *A. keiskei* than that in the other three species (*P* < 0.05). The relative contents of alcohol and ester were significantly higher in *A. keiskei* than those in *A. biserrate* and *A. dahurica* (*P* < 0.05), but there was no significant difference between *A. sinensis* and the other three species. In the comparison of heterocyclic compounds, the relative content in *A. keiskei*, *A. sinensis* and *A. biserrate* were significantly higher than the one observed in *A. dahurica* (*P* < 0.05).

### 3.3. Differential metabolites between *A. sinensis* and the three other *Angelica* species

To further identify the differential metabolites of the four *Angelica* species, we used multivariate statistical methods with |Log2FC|≥1 and VIP≥1. As *A. sinensis* was widely utilized in prescriptions of TCM (Yeh et al., 2011) and PC1 mainly differentiated *A. sinensis* from the other three species (Fig. 1b), the comparison was
conducted between *A. sinensis* and the other three species. Interestingly, there were fewer up-regulated metabolites in *A. sinensis* when compared to the other species. And no significantly enriched pathway was detected in the KEGG enrichment results of these differential metabolites (*P*-values were showed in Table S3.), which could be a bias caused by the small dataset. Compared to *A. dahurica*, 546 significantly differential metabolites (212 up-regulated and 334 down-regulated) were detected in *A. sinensis* (Fig. 4a), and the top 3 enrichment pathways of these metabolites were tyrosine metabolism (3 metabolites with *P* = 0.21), limonene and pinene degradation (5 metabolites with *P* = 0.23) and metabolic pathways (23 metabolites with *P* = 0.24) (Fig. 4d). Compared to *A. biserrate*, 558 significantly differential metabolites (155 up-regulated and 403 down-regulated) were screened in *A. sinensis* (Fig. 4b), and the top 3 enrichment pathways of these substance were metabolic pathways (24 metabolites with *P* = 0.17), tyrosine metabolism (3 metabolites with *P* = 0.23) and limonene and pinene degradation (5 metabolites with *P* = 0.25) (Fig. 4e). When compared to *A. keiskei*, 644 significantly differential metabolites (136 up-regulated and 508 down-regulated) were identified in *A. sinensis* (Fig. 4c), which were the most abundant compared to the other two group, and the top 3 enrichment pathways of these metabolites were biosynthesis of various plant secondary metabolites (5 metabolites with *P* = 0.12), metabolic pathways (26 metabolites with *P* = 0.12), and monoterpenoid biosynthesis (9 metabolites with *P* = 0.21) (Fig. 4f). In order to delve into the details of the volatile metabolite difference between *A. sinensis* and the other three species, the most significantly twenty metabolites (the top
10 for up-regulation and down-regulation, respectively) were selected (Fig. 5). It was discovered that hippuric acid, 7-hydroxycoumarin and 7-ethoxycoumarin were more enriched in *A. sinensis* than the three other *Angelica* species. In addition, the abundance of 3-butylibenzofuran-1(3H)-one in *A. sinensis* was also substantially higher than that in *A. dahurica* and *A. keiskei* (log2FC > 19). Meanwhile, the metabolites γ-terpinene, 4-hydroxyphenylacetic acid, and cinnamic acid in *A. dahurica*, and the metabolites γ-terpinene, bornyl acetate in *A. keiskei* and *A. biserrate* were in high abundance, but the metabolites were lower in *A. sinensis*.

### 3.4. Differential metabolites between *A. keiskei* and the three other *Angelica* species

Given the abundance of metabolites in *A. keiskei*, the differences of metabolites between *A. keiskei* and the other *Angelica* species were further compared. The volcanic map visually showed the overall distribution of differential metabolites in each comparison. Six hundred and four significantly different metabolites (529 up-regulated and 75 down-regulated) were detected in the comparison between *A. keiskei* and *A. dahurica* (Fig. 6a), which were associated with sesquiterpenoid and triterpenoid biosynthesis (8 metabolites with *P* = 0.15), monoterpenoid biosynthesis (9 metabolites with *P* = 0.25) and metabolic pathways (25 metabolites with *P* = 0.37) (Fig. 6c). Five hundred and seventeen significantly different metabolites (395 up-regulated and 122 down-regulated) were detected in the comparison between *A. keiskei* and *A. biserrate* (Fig. 6b), which were related to phenylpropanoid biosynthesis (2 metabolites with *P* = 0.21), metabolic pathways (17 metabolites with *P* = 0.41) and tyrosine metabolism (2 metabolites with *P* = 0.44) (Fig. 6d).
Moreover, to further investigate the differences of volatile metabolites in *A. keiskei* and other Angelica species, we subsampled twenty metabolites that were differentiated the most between the two species (Fig. 7). From the comparison, we found that carene, bornyl acetate and isobornyl acetate were the most enriched in *A. keiskei* compared to *A. dahurica*; and the terpenoids metabolites, carvenone and cedrene were more abundant in *A. keiskei* than that in *A. biserrate*. Additionally, the β-pinene was more enriched in *A. dahurica* and *A. biserrate* than in *A. keiskei*.

**Fig. 7.** Top 20 metabolites with significant difference between *A. keiskei* and *A. dahurica* (a), *A. keiskei* and *A. biserrate* (b). Red and green represent up-regulated and down-regulated metabolites in *A. keiskei*, respectively.

### 4. Discussion

Widely targeted metabolomics offered a promising way for the chemical screening of volatile metabolites and allowed the characterization of new volatile metabolites in *Angelica* (Kumar et al., 2022). Using the method, a total of 899 volatile metabolites were identified and further classified into 16 different categories, including terpenoids, ester, heterocyclic, aromatics and 12 others (Fig. 2). A clustering heat map of the metabolites showed significant difference among the four species. The number of types and abundance of volatile metabolites in *A. keiskei* was the highest. Consistent with the previous reports, terpenoids were the largest and most diverse class of volatile metabolites in the four *Angelica* species (Abbas et al., 2017; Sowndhararajan et al., 2017). The pair wise comparisons between two species for the metabolite’s differences revealed that there were fewer up-regulated metabolites in *A. sinensis*.
when compared to the other three species (*A. dahurica*, *A. keiskei*, *A. biserrate*)

whereas, relative to *A. keiskei*, most differential metabolites were down-regulated in *A. dahurica* and *A. biserrate*. It demonstrates that the analysis of differential metabolites is useful for understanding the differences of chemical properties among the four species.

*Angelica sinensis* also known as “female ginseng” is a traditional herb, which has long been used to treat various gynecological conditions (Hook, 2014; Wei et al., 2016). Z-ligustilide is believed to be responsible for the bioactivities of *A. sinensis* (Chen et al., 2013; Wei et al., 2016). This study shows that Z-ligustilide was detected in the four species and its contents were relatively higher in *A. sinensis*, which is consistent with previous studies (Hook, 2014). In addition, Coumarin and its derivatives are one of the important heterocyclic metabolites (Wu et al., 2009), which is mainly used as anti-HIV, anticancer activity agents, and anticoagulant activities (Kim et al., 2022; Zhou et al., 2016). We observed that the contents of 7-hydroxycoumarin and 7-ethoxycoumarin in *A. sinensis* were significantly higher than *A. dahurica*, *A. biserrate*, and *A. keiskei* (Fig. 2). By virtue of its structural simplicity, 7-hydroxycoumarin has been generally accepted as the parent metabolites for the furocoumarins and pyranocoumarins and is widely used as a synthon for a wide variety of coumarin-heterocycles (Han et al., 2022; Mazimba, 2017; Vanholme et al., 2019). Its higher abundance in *A. sinensis* was probably associated with biosynthesis of furocoumarins and pyranocoumarins, which were reported as one of the main active components influencing the pharmaceutical activity of the herb.
Nevertheless, in ancient Chinese medical systems, the pharmacological effect of medicinal plants depends not only on the high abundance of a single compound, but also on the synergy of multiple active ingredients (Liu et al., 2014; Song et al., 2016). Furthermore, this study also found that the proportion of various components in volatile metabolites was more balanced in *A. sinensis* (Fig. 2). This might explain the wide and common applications of *A. sinensis* in TCM. Meanwhile, the results showed that the abundance of volatile metabolites in the root of the *A. keiskei* was the highest among the four species. It has been used as a medicine and food owing to its abundant pharmacological effects, including anti-cancer, lowering blood sugar and blood lipids, and improving human immunity (Guiné and Gonçalves, 2016; Kil et al., 2017). However, these pharmacological effects have not been validated in scientific research. To date, it is only found in the form of raw materials in tea and cosmetics, which has limited its medicinal and clinical applications (Kim et al., 2014; Rong et al., 2021). Interestingly, bornyl acetate, previously unmentioned terpenoid substances was detected with high expression levels in the root of *A. keiskei*, and it has been reported that bornyl acetate has antibacterial, insecticidal, and anesthetic effects symbiotically with other aromatic metabolites in the VOs (Liang et al., 2022). This discovery provides a basis for the development and utilization of active ingredients in *A. keiskei* for health-related dietary supplements. Taken together, this study greatly enriches the database of chemical composition in *A. keiskei* and imply that *A. keiskei* exhibited benign
potential to be exploited as medicinal materials and health-related dietary

Previous studies have verified that plants with closer phylogenetic relationship are not only similar in morphology but also in chemical composition and curative effects (Hao and Xiao, 2020; Kang et al., 2019; Saslis-Lagoudakis et al., 2011). Here, this study performed hierarchical clustering analysis based on Bray-Curtis’s dissimilarity distances of the composition and abundance of volatile metabolites in the four

Angelica species. The dendrogram (Fig. 8a) showed high correspondence with the phylogenetic tree (Fig. 8b) based on chloroplast sequences, suggesting a correlation relationship between the volatile metabolites and the phylogenetic relationships. Although more extensive sampling and deeper investigations would be necessary to reveal more reliable correlations, the study implied that phylogenetic relationships could serve as a window to coarsely apprehend the unknown biochemical diversity of some plants based on the known biochemical map of phylogenetically related species. This finding may offer a great tool for searching replacements of medicinal plant resources that are endangered with closely related non-endangered species.

5. Conclusion

This study investigated the metabolites of four Angelica species by using widely targeted metabolomics, and found the differed accumulation of medicinally important metabolites among species. For example, high levels of bornyl acetate metabolites accumulated in A. keiskei, whereas coumarins and phthalides were significantly lower in A. keiskei than in A. sinensis. Moreover, the high correspondence between the

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dendrogram of metabolite contents and the phylogenetic tree suggested a potential
correlation between the volatile metabolites and the phylogenetic relationships. Taken
all together, we are convinced that the present study provides a biochemical map for
the exploitation, application, and development of the *Angelica* species as TCM or
health-related dietary supplements.

**Credit authorship contribution statement**

Li Wang conceived and designed the study. Lan-Lan Zang, Jiao-Jiao Ji, Ting-Ting Lu
and Xiao-Xu Han prepared the materials. Jiao-Jiao Ji and Lan-Lan Zang performed
data analyses. Lan-Lan Zang and Jiao-Jiao Ji wrote the first version of the manuscript
with suggestions from Li Wang. Li Cheng, Xiao-Xu Han, Soorang Lee, Lei Ma and
Li Wang revised the manuscript. All authors read and approved the final manuscript.
Lan-Lan Zang and Jiao-Jiao Ji contributed equally to this work.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal
relationships that could have appeared to influence the work reported in this paper.

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (Grant
No. 32070242), the National Key Research and Development Program of China
(Grant No. 2020YFA0907900), the Shenzhen Science and Technology Program
(Grant No. KQTD2016113010482651), special funds for Science Technology
Innovation and Industrial Development of Shenzhen Dapeng New District (Grant Nos.
RC201901-05 and PT201901-19), the China Postdoctoral Science Foundation (Grant
No. 2020M672904), and the Basic and Applied Basic Research Fund of Guangdong (Grant No. 2020A1515110912).

Data availability

All study data are included in the article and supporting information.
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Fig. 1. An overview of volatile metabolites among four *Angelica* species. (a) Venn diagram showing the number of common and specific metabolites in the four species. (b) PCA of volatile metabolites for the four species with three biological replicates. (c) Heatmap clustering of volatile metabolites identified from the four species. Volatile metabolite abundance was z-score transformed.

Note: The color-coded scale grading from green to red corresponds to the content of volatile metabolites shifting from low to high.
Fig. 2. Classification and proportion of volatile metabolites detected in the four Angelica species. (a) *A. sinensis*, (b) *A. dahurica*, (c) *A. biserrate*, (d) *A. keiskei*. 
Fig. 3. Comparison for the relative abundance of seven categories (alcohol, aromatics, aldehyde, ester, heterocyclic compounds, ketone and terpenoids) with significant differences in the four Angelica species.
Fig. 4. The overall distribution and KEGG enrichment analysis of differential metabolites between *A. sinensis* and the three other *Angelica* species. (a-c) Volcano plots for differential metabolites between *A. sinensis* and the three other *Angelica* species. (a) *A. sinensis* vs *A. dahurica*. (b) *A. sinensis* vs *A. biserrate*. (c) *A. sinensis* vs *A. keiskei*. Colors of metabolites indicated significant differences (red, upregulated; green, downregulated). (d-f) KEGG pathway enrichment analysis of differential metabolites for *A. sinensis* vs *A. dahurica* (d), *A. sinensis* vs *A. biserrate* (e) and *A. sinensis* vs *A. keiskei* (f). Note: Color of the bubbles represented statistical significance of the enriched terms, and the size of the bubbles represented number of differentially enriched metabolites. The pathway of...
“Biosynthesis of various plant secondary metabolites” including: crocin biosynthesis, cannabidiol biosynthesis, mugineic acid biosynthesis, pentagalloylglucose biosynthesis, benzoxazinoid biosynthesis, gramine biosynthesis, coumarin biosynthesis, furanocoumarin biosynthesis, hordatine biosynthesis, podophyllotoxin biosynthesis.
Fig. 5. The top 20 metabolites of significantly differential volatiles between *A. sinensis* and three other *Angelica* species. Red indicated the more abundant metabolites in *A. sinensis* compared to *A. dahurica* (a), *A. biserrate* (b), *A. keiskei* (c). Green indicated the lower levels of metabolites in *A. sinensis* than that in other species.
Fig. 6. The overall distribution and KEGG enrichment analysis of differential metabolites between A. keiskei and A. dahurica (a, c), A. keiskei and A. biserrate (b, d). (a-b) Volcano plots for differential metabolites. The colors of metabolites indicated significant differences (red, upregulated; green, downregulated). (c-d) KEGG pathway enrichment analysis of differential metabolites.

Note: Color of the bubbles represented statistical significance of the enriched terms, and the size of the bubbles represented number of differential metabolites.
Fig. 7. Top 20 metabolites with significant difference between A. keiskei and A. dahurica (a), A. keiskei and A. biserrate (b).

Note: Red and green represent up-regulated and down-regulated metabolites in A. keiskei, respectively.
Fig. 8. Hierarchical clustering based on the similarity of volatile metabolites (a) and phylogenetic tree of the four Angelica species and H. sibthorpioides (b). The chloroplast sequences above were available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov].