

Metabolomics analysis reveals the differences between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd

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Abstract

Bupleurum chinense DC. and *Bupleurum scorzonerifolium* Willd. are two varieties of *Bupleuri radix* in Chinese Pharmacopoeia 2020. Clinical efficacy of the two bupleurum species is different. Detailed comparisons of metabolites between the two varieties and different tissues are not available. In order to analyze the difference of metabolites, we used LC-MS and GC-MS to detect the root, stem, leaf and flower of two varieties, and detected 1818 metabolites in 25 classes. We performed statistical analysis of metabolites. Differential metabolites were screened by Fold Change and VIP values of OPLS-DA model and significant differences were found among different groups. The active components triterpenoid saponins in BcR were more than BsR. Other pharmacological substances were significantly different. By KEGG annotation and enrichment analysis, we found that the differential metabolites of the aboveground part mainly concentrated in monoterpenoids biosynthesis, while the differential metabolites of the root mainly concentrated in sesquiterpenoid and triterpenoid biosynthesis. Differences in metabolic networks may indirectly affect the metabolic profile of Bc and Bs, leading to differences in clinical efficacy. Our study maybe provide reference for evaluating the quality of medicinal materials, developing other medicinal parts and guiding the clinical use of traditional Chinese medicine.

1. Introduction

Plants, as direct or indirect sources of nutrition, energy and medicine for human beings, can synthesize a large number of metabolites with different biological functions under the changeable environmental stimulation. In the long evolutionary process, plants constantly mutate in order to adapt to changeable environments.¹ They occupying wide habitats usually vary in phenotypes such as in morphology and metabolism, thereby developing into different ecotypes. Plants contain primary and secondary metabolites. The latter are involved in the environmental response of resistance to disease and stress, and some of substances have important medicinal values for humans.² On a global scale, we recognize the importance of medicinal plants for humanity. They can supplement the nutrition needed by the human body, improve the immunity of the human body, improve the state of disease, and even fight epidemics, such as SARS-CoV-2³ and malaria⁴. According to WHO reports, around 80 % of the global population still relies on botanical drugs.⁵ Natural substances have long served as sources of therapeutic drugs.

Bupleurum species are found mainly in the subtropical regions of the Northern hemisphere⁶. The dry roots are used as herbal medicine in many countries, and some species have been officially listed in the Chinese, Japanese, Korean, British and European Pharmacopoeias.⁷⁸ These species are used either alone or in combination with other ingredients for treatment of the common cold, inflammatory disorders, hepatitis and fever.⁹ Up to now, phytochemical studies have shown *Bupleurum* contains abundant natural compounds, such as flavonoids, lignins, phenolics, phenyl propanol derivatives, triterpenoid saponins, and volatile oils.¹⁰¹¹ In the 2020 edition of the Chinese Pharmacopoeia, *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. were included as the two authentic sources of Chinese traditional

medicine *Bupleuri Radix* (chaihu). *Bupleuri Radix* has been recorded in the Shennong Traditional Herbal Scriptures under its original name of Purput and was listed among the top herbs. It was renamed *Bupleurum* in the Book of Materia Medica and has been used for more than 2,000 years.¹²¹³ This herb is known to disperse and reduce fever, relieve liver depression and lift yang-qi. The main active components of *bupleurum* are triterpene saponins, and specifically saikosaponins (SSs)¹⁴ as a research hotspot.¹⁵¹⁶ In China from ancient to present, the efficacy of the two *bupleurum* species is different. Shiyuan Jin, a master of traditional Chinese medicine, said the use of two types of *bupleurum* in Beijing required doctors to write prescriptions separately according to their needs. *Bupleurum chinense* DC. commonly known as Northern *Bupleurum* is often used to treat typhoid fever. *Bupleurum scorzonerifolium* Willd. commonly known as Southern *Bupleurum* is often used to Clear liver heat, so they are different in clinical use. The difference in clinical efficacy is closely related to the composition of plant metabolites.

Now demand for medicinal plants is in short supply. In order to improve the utilization of plants and alleviate resource shortages, researchers develop other tissue parts of medicinal plants¹⁷. Different tissue parts have different composition, and the curative effect is different.¹⁸¹⁹ The official medicinal part of *bupleurum* is the root. "Qianjin Yifang" recorded that the use of *Bupleurum* seedlings treating sudden deafness, so the stems and leaves of *bupleurum* were also used at that time, and their effects were different from root. Now the standard of local medicine in China includes the whole herb of *Bupleurum scorzonerifolium* Willd.. Studies have found that the flower of *Bupleurum chinense* DC. contain a lot of flavonoids.²⁰ The development of aboveground part of *bupleurum* is beneficial to saving resources and protecting ecology. This study compared the metabolomics composition of different tissue parts of *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd., so as to provide reference for evaluating the quality of medicinal materials, developing other medicinal parts and guiding the clinical use of traditional Chinese medicine.

2. Materials And Methods

2.1 Plant Materials. In this study, 12 wild *Bupleurum chinense* DC. (Bc) and 12 wild *Bupleurum scorzonerifolium* Willd. (Bs) (At least one year old) were selected from suburban mountainous areas of Beijing, China in July 2021. After the plants were harvested, rinsed with running water, then rinsed with sterile water. The roots(R), stems(S), leaves(L) and flowers(F) were separated with a blade on a clean table, sampled, and stored at -80°C until further analysis. A total of eight groups (BcR, BcS, BcL, BcF, BsR, BsS, BsL, BsF), each sample had three biological replicates, and each repeat contains three individual plants. Fewer samples were taken because there were fewer wild *bupleurum* in the mountain.

2.2 Sample Preparation and Extraction. The samples were dried in vacuum freeze-drying machine (ScientZ-100F). The tissue was ground to powder by using a grinder (MM 400; Retsch, Haan, Germany) for 1.5 min at 30 Hz. Then, weigh 100 mg powder, dissolve it in 1.2 mL 70% aqueous methanol and vortexed 6 times for 30 seconds each, overnight at 4 °C. After centrifugation at 12,000 g for 10min, the

supernatant was absorbed, the sample was filtered by microporous membranes (0.22 µm pore size, ANPEL, Shanghai, China) and stored in vials followed by UPLC –MS analysis.²¹²²²³

Another part of the sample was ground with liquid nitrogen and mixed evenly by vortex. Each sample was weighed about 1 g in a headspace bottle. Add saturated NaCl solution and 10 µL (50 µg/mL) internal standard solution, respectively. Extraction was carried out by automatic headspace solid phase microextraction (HS-SPME) for GC-MS analysis.

2.3 Ultra Performance Liquid Chromatography and Gas Chromatography Conditions. The extracts were analyzed by LC-ESI-MS/MS system (HPLC, UFLC SHIMADZU Nexera X2; MS, 4500 QTRAP; Applied Biosystems, Foster City, CA, USA) equipped with a C18 column (Agilent SB-C18 2.1 mm × 100 mm, 1.8 µm). The mobile phase included ultra-pure water (A) and acetonitrile (B) with 0.1% acetic acid. Perform the elution gradient procedure as follows: 95:5 V/V for 0 min, 5:95 V/V at 9.0 min, 5:95 V/V at 10.0 min, 95:5 V/V at 11.1 min, and 95:5 V/V at 14.0min. The flow rate was 0.35 mL/min. The column temperature was set at 40 °C, and the injection volume was 4 µL. The effluent was connected to an ESI-triple quadrupole linear ion trap (Q TRAP)-MS.²⁴

Desorption of the volatile organic chemicals (VOCs) from the fibre coating was carried out in the injection port of the GC apparatus (Model 8890; Agilent) at 250°C for 5 min in the splitless mode. GC apparatus equipped with a DB-5MS capillary column (30 m × 0.25mm × 0.25 µm, Agilent J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a linear velocity of 1.2 mL/min. The injector temperature was kept at 250°C and the detector at 280°C. The oven temperature was programmed from 40°C (3.5 min), increasing at 10°C/min to 100°C, at 7°C/min to 180°C, at 25°C/min to 280°C, hold for 5 min. The effluent was connected to an electron impact (EI)-MS.

2.4 ESI-Q TRAP-MS/MS and EI-MS conditions. In order to detect metabolites, AB4500 Q TRAP LC/MS/MS system equipped with linear ion trap (LIT) and triple quadrupole (QQQ) scans was operated. This system was equipped with an ESI turbo ion spray interface operating in positive and negative ion monitoring mode controlled by Analyst v1.6.3 software (AB Sciex, Foster City, CA, USA). The ESI source was an ion source (turbo spray, 550 °C, 5500 V). Gas I, gas II, and curtain gas (CUR) were set at 50, 60, and 25 psi, respectively. The collision gas was set to high. In the QQQ and LIT modes, 10 and 100 µmol/L polypropylene glycol solutions, respectively, were used for the instrument tuning and mass calibration. QQQ scans were obtained in the multiple reaction monitoring (MRM) experiments with collision gas (nitrogen) set to 5 psi. By further de-clustering potential (DP) and collision energy (CE) optimization, the DP and CE of each MRM ion pair were completed. According to the eluted metabolites in each period, a specific set of MRM transitions was monitored.

GC-MS was recorded in electron impact (EI) ionisation mode at 70 eV. The quadrupole mass detector, ion source and transfer line temperatures were set, respectively, at 150, 230 and 280°C. The MS was selected ion monitoring (SIM) mode was used for the identification and quantification of analytes.

2.5 Qualitative and Quantitative Metabolite Analyses. We compared the accurate precursor ions (Q1), product ion (Q3) values, retention times (RT), and fragmentation patterns with standards to analyze the primary and secondary MS data, when standards were available (Sigma-Aldrich, St. Louis, MO, USA). When standards were unavailable, we used the MWDB (MetWare Biological Science and Technology Co., Ltd., Wuhan, China) which is a self-compiled database to analyze the data. Qualitative analysis was carried out according to secondary spectrum information, and isotope signals, repeated signals containing K⁺, Na⁺, NH₄⁺, and repeated signals of fragments of other substances with larger molecular weight were removed during analysis.

The quantitative analysis was carried out in the MRM mode. The characteristic ions of each metabolite were screened through the QQQ MS to obtain the signal strengths. Integration and correction of chromatographic peaks were conducted using MultiQuant v3.0.2 (AB Sciex, Concord, Ontario, Canada). The corresponding relative metabolite contents were expressed as chromatographic peak area integrals.

We used a self-compiled database MWGCSIM1.0 ((MetWare Biological Science and Technology Co., Ltd., Wuhan, China) for GC-MS. Each compound matched a quantitative ion and 2 ~ 3 qualitative ion. The retention time of the detected chromatographic peak was consistent with the standard reference, and all the selected ions appeared in the sample quality spectrum after deducting the background, which was determined as the substance²⁵. Quantitative ions were selected to integrate and correct chromatographic peaks to enhance the accuracy of quantification. Integration and correction of chromatographic peaks were conducted using MassHunter.

In order to keep the experimental data stable, we carried out sample quality control analysis (Figure 1A-D) before testing the sample.

2.6 Multivariate Statistical Analysis. Metabolite data were log₂-transformed for statistical analysis to improve normality and were normalized. Metabolites from 24 samples were used for hierarchical clustering analysis (HCA) within R (ComplexHeatmap 2.8.0), principal component analysis (PCA) within R (base package 3.5.1), and orthogonal partial least squares discriminant analysis (OPLS-DA) within R (MetaboAnalystR 1.0.1). Venn diagrams were used to illustrate the number of differential metabolites. Identified metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Compound database (<http://www.kegg.jp/kegg/compound/>), annotated metabolites were then mapped to KEGG Pathway database (<http://www.kegg.jp/kegg/pathway.html>).

3. Results

3.1 Morphological Differences. *Bupleurum chinense* DC. and *Bupleurum scorzonrifolium* Willd. (Figure 2) grown in the same season and under the conditions. The morphology particularly the roots was obviously different. The roots of Bc have many branches, and the branches of Bs are few. The skin color of Bc is chocolate-brown, but Bs is reddish-brown.

3.1 Morphological Differences. *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. (Figure 2) grown in the same season and under the conditions. The morphology particularly the roots was obviously different. The roots of Bc have many branches, and the branches of Bs are few. The skin color of Bc is chocolate-brown, but Bs is reddish-brown.

3.3 Multivariate Analysis Revealed Differences among the Metabolite Profiles. Multivariate Statistical Analysis were performed to assess the differences among the metabolite profiles of 8 groups. The results of HCA showed that there were significant differences in different groups, which were divided into four clusters (Figure 4A). The metabolites in cluster 1 were highest in BsF and BsL, cluster 2 in BcF and BsF, cluster 3 in BsS and BcL, and cluster 4 in BcR and BsR. It also clustered between biological replicates, indicating good homogeneity between biological replicates and reliability of data.

PCA was used to uncover the internal structure of multiple variables through several principal components (Figure 4D). In the PCA diagram (Figure 4B-C), quality control (QC) samples is the mixture of bupleurum sample extracts, were projected to the same area and some even overlapped, so the analysis was stable and repeatable. This result showed that the tissue sites were separated on the first principal component and the varieties had obvious separation on the second principal component. There was obvious grouping tendency among different tissues of different varieties.

In general, HCA and PCA showed that there were significant differences in metabolites in different groups, with BcR and BsF showing the biggest differences.

3.4 Differential Metabolite Analysis. Pairwise comparisons were conducted among 8 groups (Figure 5D). In the OPLS-DA models (Figure 5A-B), significant segregation occurred in all comparison groups.

We further performed different metabolite screening based on the fold-change ($FC \geq 2$ or ≤ 0.5) and variables identified as important in the projection scores ($VIP > 1$). The screening results are presented as volcano plots (Figure 5C) and Venn diagrams (Figure 6B).

In all comparison groups, BcR and BsR were down-regulated the most, which was consistent with the result of cluster analysis. We paid special attention to the differential metabolites in the medicinal part root group. There were 759 differential metabolites, among which 247 metabolites were up-regulated and 512 metabolites were down-regulated. There were 150 terpenoids in the differential metabolites, accounting for about 20% (Table 1).

Table 1 Classification and quantity of differential metabolites between BcR and BsR

Type	Number	Percentage(%)
Terpenoids	150	19.8
Phenolic acids	73	9.6
Flavonoids	72	9.5
Lipids	44	5.8
Lignans and Coumarins	43	5.7
Hydrocarbons	27	3.6
Alkaloids	15	2.0

In the group of BcR and BsR, seven of the top ten down-regulated saponins were saikosaponins (Figure 6A) and the specific of them were Saikosaponin A, Prosaikogenin G, Saikosapanin B1, Saikosaponin G, Saikosaponin C, Saikosaponin L, Saikosaponin BK1. Basically all saponins metabolites of Bs were down-regulated, indicating that the content of Saikosaponins in the root of Bc was relatively high. The top ten down-regulated metabolites had lignans, phenolic acids and the volatile organic chemicals. In BsR, 6-O-Galloyl-D-glucose which has antihypertensive activity more than BcR²⁶. More saikosaponins (SSA,SSC,SSD,SSB4,SSF), which have been shown to have antidepressant and anti-inflammatory effects, were detected in BcR than in BsR. Compared with BcR, lignans, phenolic acids and the volatile organic chemicals were higher in BsR. In terms of isorhamnetin, a heart-protecting substance, we found 16 isorhamnosides were more accumulated in BsR than in BcR.

In the comparison between BcS and BsS, there were 729 differential metabolites in total, among which 423 metabolites were up-regulated and 306 metabolites were down-regulated, and flavonoids were the most different substances. In the comparison between BcL and BsL, there were 844 differential metabolites in total, among which 641 metabolites were up-regulated and 203 metabolites were down-regulated, and flavonoids and terpenoids were mainly different substances. In the comparison between BcF and BsF, there were 722 differential metabolites in total, among which 452 metabolites were up-regulated and 270 metabolites were down-regulated. In BcF, hispidulin-8-C-(2"-O-xylosyl)glucoside were found more than BsF. In BsF, kaempferol-3-O-glucuronide which had been found to treat non-alcoholic steatohepatitis²⁷ were found more than BcF.

We also compared the differential metabolites between different tissue parts. In contrast group of BcR and BcF, 841 metabolites were up-regulated, including 149 flavonoids. In Bc, there are more triterpenoid saponins in roots, but more flavonoids and volatile components in flowers. In contrast group of BsR and BsF, 966 metabolites were up-regulated including 109 flavonoids and 78 phenolic acids, and the number of up-regulated metabolites was the largest in all contrast groups. So we can develop flowers of Bc and Bs to use active ingredients such as flavonoids in flowers. In addition, there were more flavonoids and phenolic acids in the aboveground part than in the root of Bs.

We identified 144 common differential metabolites from Venn diagrams. The common differential substance indicated that Bc and Bs had differences in roots, stems, leaves and flowers, belonging to interspecific differential substance. We focused on triterpenoid saponins in all down-regulated components in four contrast groups, such as Oleanolic acid-3-O-xylosyl(1→3)glucuronide, Oleanolic acid-3-O-glucosyl(1→2)glucoside, Bupleurumwenchuanense, saikosaponin K, Rotundifolioside A, Rotundifolioside H, Medicagenic acid-3-O-glucuronide-28-O-rhamnosyl(1,2)-arabinoside. More triterpenoid saponins were synthesized in Bc than Bs, which was closely related to the expression of genes related to triterpenoid saponins synthesis pathway. The other substances all down-regulated most were flavones, flavonoid carbonoside and flavonols, including 8 kinds of kaempferitrins and apiins. All up-regulated substances included 25 flavonoids, 11 phenolic acids, as well as some volatile components.

It is noteworthy that shikimic acid, as the prerequisite of phenylpropane pathway, was lower in all parts of Bs than that of Bc, which may be related to the metabolism of phenylpropane pathway in the two varieties. Isoscopoletin (6-Hydroxy-7-Methoxycoumarin), which had antiasthmic effect, was higher in Bc than Bs.

3.5 KEGG annotation and enrichment analysis. The differential metabolites among the eight accessions were mapped to the KEGG Pathway database to obtain detailed pathway information (Figure 6.A-F). Differential metabolites of BcR and BsR are mainly annotated and enriched in sesquiterpenoid and triterpenoid biosynthesis, flavonoid biosynthesis, and biosynthesis of secondary metabolites. Differential metabolites of BcS and BsS are mainly annotated and enriched in flavone and flavonol biosynthesis, limonene and pinene biosynthesis, monoterpene biosynthesis, and folate biosynthesis. Differential metabolites of BcL and BsL are mainly annotated and enriched in monoterpene biosynthesis, limonene and pinene biosynthesis, and biosynthesis of secondary metabolites. Differential metabolites of BcF and BsF are mainly annotated and enriched in monoterpene biosynthesis, flavone and flavonol, and biosynthesis of secondary metabolites.

Some metabolic pathways overlap in these comparison groups, such as biosynthesis of secondary metabolites and monoterpene biosynthesis. The differential metabolites of the aboveground part of the two species mainly concentrated in the biosynthesis pathway of monoterpenoids, while the differential metabolites of the root mainly concentrated in the sesquiterpenoid and triterpenoid biosynthesis. The difference in function between the two species can be explained by pathway, which is mainly due to the difference in terpenoids.

Differential metabolites of BcR and BcF are mainly annotated and enriched in flavonoid biosynthesis, flavone and flavonol biosynthesis, pyrimidine metabolism, zeatin biosynthesis, sesquiterpenoid and triterpenoid biosynthesis. Flavonoid biosynthesis pathway in flowers deserves attention.

4. Discussion

The dry roots of *Bupleurum* are used as herbal medicine in many countries²⁸¹. *Bupleuri Radix*, as a traditional bulk medicinal material in China, its main effective components are saikosaponins, which have anti-cancer, anti-inflammatory and anti-depression effects. *Bupleurum chinense* DC. and *Bupleurum scorzonrifolium* Willd. were included as the two authentic sources of Chinese traditional medicine *Bupleuri Radix* in the Chinese Pharmacopoeia 2020. However, there were some differences in phenotype and clinical efficacy between the two varieties. Studies have shown that bupleurum overground parts also have certain medicinal effects, and the development of non-medicinal parts is beneficial to saving resources and protecting ecology. The metabolomics analysis of different tissue parts of Bc and Bs can provide reference for evaluating the quality of medicinal materials, developing other medicinal parts and guiding the clinical use of traditional Chinese medicine.

We used LC-MS and GC-MS to detect the root, stem, leaf and flower of Bc and Bs and detected a total of 1818 metabolites in 25 classes. 154 terpenoids were detected, including 30 triterpene Saponin, 19 triterpenoids and 11 monoterpenoids. We performed statistical analysis of metabolites, and significant differences were found among different groups. We also focused on some of the more diverse pharmacological components.

The active components triterpenoid saponins in BcR were more than those in BsR, while lignans, phenolic acids and the volatile organic chemicals were higher in BsR. In BsR, 6-O-Galloyl-D-glucose which has antihypertensive activity more than BcR. The triterpenoid saponins in BcR were more than those in BcF, while the flavonoids and volatile organic chemicals in BcF were more. In BcF, hispidulin-8-C-(2"-O-xylosyl)glucoside were found more than BsF. In BsF, kaempferol-3-O-glucuronide which had been found to treat non-alcoholic steatohepatitis were found more than BcF. The results showed that 7 triterpenoid saponins were more accumulated in each part of Bc than that of Bs. The aboveground part is rich in active ingredients and has great value for development and utilization. By KEGG annotation and enrichment analysis, we found that the differential metabolites of the aboveground part of the two species mainly concentrated in the biosynthesis pathway of monoterpenoids, while the differential metabolites of the root mainly concentrated in the sesquiterpenoid and triterpenoid biosynthesis.

Differential metabolites of different *Bupleurum* species involve many different metabolic pathways, and these metabolic pathways depend on each other to maintain the physiological activities of organs. For example, terpene biosynthesis has been reported to be associated with glycolysis and the pentose phosphate pathway²⁹². Mevalonic acid biosynthesis provides a precursor substance for terpene biosynthesis³⁰³. The differences in metabolic networks may firsthand affect the metabolic profile of Bc and Bs, leading to differences in clinical efficacy. Different tissues of plants also play different functions, and their metabolic pathways are also different. The differences between tissues are related to the differential expression of genes and also involve the transport and accumulation of metabolites between different tissues.

Abbreviations

BcR the root of *Bupleurum chinense* DC.

BsR the root of *Bupleurum scorzonerifolium* Willd.

BcS the stem of *Bupleurum chinense* DC.

BsS the stem of *Bupleurum scorzonerifolium* Willd.

BcL the leaf of *Bupleurum chinense* DC.

BsL the leaf of *Bupleurum scorzonerifolium* Willd.

BcF the flower of *Bupleurum chinense* DC.

BsF the flower of *Bupleurum scorzonerifolium* Willd.

CV Coefficient of Variation

CPS Count per second

GC-MS Gas chromatography coupled with mass spectrometry

HCA hierarchical clustering analysis

KEGG Kyoto encyclopedia of genes and genomes

LC-MS Liquid chromatography coupled with mass spectrometry

MS Mass spectrometry

OPLS-DA Orthogonal projections to latent structures-discriminate analysis

PCA Principal component analysis

QC Quality control

RT Retention time

UHPLC Ultra high performance liquid chromatography

VIP Variable importance in the projection

Declarations

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Author contributions

QXJ and LCL conceived and designed the experiments. QXJ, HSQ, LT and LCL collected the plant material and performed the experiments. QXJ and LCL analyzed the data. QXJ and LCL wrote the article. QXJ, HSQ, LT, ZJQ, WBS and LCL have discussed the results and contributed to the drafting of the manuscript. All authors read and approved the final version of the manuscript.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Additional Information

The authors declare no competing interests.

References

1. Brunetti C, George RM, Tattini M, Field K, Davey MP. Metabolomics in plant environmental physiology. *J Exp Bot.* 2013 Oct;64(13):4011-20.
2. Yang L, et al. Response of plant secondary metabolites to environmental factors. *Molecules.* 2018 Mar 27;23(4):762.
3. Wang Z, Yang L. Chinese herbal medicine: Fighting SARS-CoV-2 infection on all fronts. *J Ethnopharmacol.* 2021 Apr 24;270:113869.
4. Talman AM, Clain J, Duval R, Ménard R, Arieu F. Artemisinin Bioactivity and Resistance in Malaria Parasites. *Trends Parasitol.* 2019 Dec;35(12):953-963.
5. Sen T, Samanta SK. Medicinal plants, human health and biodiversity: a broad review. *Adv Biochem Eng Biotechnol.* 2015;147:59-110.
6. Huang R, Xie X, Chen A, Li F, Tian E, Chao Z. The chloroplast genomes of four *Bupleurum* (Apiaceae) species endemic to Southwestern China, a diversity center of the genus, as well as their evolutionary implications and phylogenetic inferences. *BMC Genomics.* 2021 Oct 2;22(1):714.
7. Ashour, M. L., and Wink, M. (2011). Genus *bupleurum*: a review of its phytochemistry, pharmacology and modes of action. *J. Pharm. Pharmacol.* 63, 305–321.
8. Sun T, et al. Summary on international quality standards of *Bupleuri Radix*. *China Journal of Chinese Materia Medica.* 2020, 45(20): 4853-4860.

9. Moses T, et al. Combinatorial biosynthesis of sapogenins and saponins in *Saccharomyces cerevisiae* using a C-16 α hydroxylase from *Bupleurum falcatum*. 2014 Jan 28; 111(4):1634-9.
10. Jiang H, et al. Botany, traditional uses, phytochemistry, analytical methods, processing, pharmacology and pharmacokinetics of *Bupleuri Radix*: A systematic review. *Biomed Pharmacother*. 2020 Nov;131:110679.
11. Sun P, et al. Pharmacological Effects and Chemical Constituents of *Bupleurum*. *Mini Rev Med Chem*. 2019;19(1):34-55.
12. Deborde C, Jacob D. MeRy-B, a metabolomic database and knowledge base for exploring plant primary metabolism. *Methods Mol Biol*. 2014;1083:3-16.
13. Pavlidis DE, Mallouchos A, Nychas GJ. Microbiological assessment of aerobically stored horse fillets through predictive microbiology and metabolomic approach. *Meat Sci*. 2021 Feb;172:108323.
14. Li X, Li X, Huang N, Liu R, Sun R. A comprehensive review and perspectives on pharmacology and toxicology of saikosaponins. *Phytomedicine*. 2018 Nov 15;50:73-87.
15. He Y, et al. Transcriptome and metabolome analysis to reveal major genes of saikosaponin biosynthesis in *Bupleurum chinense*. *BMC Genomics*. 2021 Nov 19;22(1):839.
16. Yu M, et al. Differential Expression of Genes Involved in Saikosaponin Biosynthesis Between *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd. *Front Genet*. 2020 Oct 16;11:583245.
17. Liu Y, et al. New flavonoids from the aerial part of *Bupleurum chinense* DC. *Fitoterapia*. 2020 Nov;147:104739.
18. Liu Y, et al. Aromatic glycosides from the aerial part of *Bupleurum chinense*. *J Asian Nat Prod Res*. 2021 Dec 22:1-8.
19. Maqbool B, et al. Ginseng stem-leaf saponins in combination with selenium enhance immune responses to an attenuated pseudorabies virus vaccine. *Microbiol Immunol*. 2019 Jul;63(7):269-279.
20. Zhang L, He YX, Yao JC, Yin WP. Studies on the Chemical Constituents of the Flowers of *Bupleurum chinense*. *Journal of Chinese Medicinal Materials*, 2010, 33(07):1086-1088.
21. Chen W, et al. A novel integrated method for large-scale detection, identification, and quantification of widely targeted metabolites: application in the study of rice metabolomics. *Mol Plant*. 2013 Nov;6(6):1769-80.
22. Peng M, et al. Differentially evolved glucosyltransferases determine natural variation of rice flavone accumulation and UV-tolerance. *Nat Commun*. 2017 Dec 7;8(1):1975.
23. Zhu G, et al. Rewiring of the Fruit Metabolome in Tomato Breeding. *Cell*. 2018 Jan 11;172(1-2):249-261.e12.
24. Zhang J, Qiu X, Tan Q, Xiao Q, Mei S. A Comparative Metabolomics Study of Flavonoids in Radish with Different Skin and Flesh Colors (*Raphanus sativus* L.). *J Agric Food Chem*. 2020 Dec 9;68(49):14463-14470.

25. Yuan H, Cao G, Hou X, Huang M, Du P, Tan T, Zhang Y, Zhou H, Liu X, Liu L, Jiangfang Y, Li Y, Liu Z, Fang C, Zhao L, Fernie AR, Luo J. Development of a widely targeted volatilomics method for profiling volatiles in plants. *Mol Plant*. 2022 Jan 3;15(1):189-202.
26. Hsu FL, Lee YY, Cheng JT. Antihypertensive activity of 6-O-galloyl-D-glucose, a phenolic glycoside from *Sapium sebiferum*. *J Nat Prod*. 1994 Feb;57(2):308-12.
27. Deng Y, et al. Kaempferol-3-O-Glucuronide Ameliorates Non-Alcoholic Steatohepatitis in High-Cholesterol-Diet-Induced Larval Zebrafish and HepG2 Cell Models via Regulating Oxidation Stress. *Life (Basel)*. 2021 May 14;11(5):445.
28. Yao RY, Zou YF, Chen XF. Traditional Use, Pharmacology, Toxicology, and Quality Control of Species in Genus *Bupleurum* L. *Chin Herb Med*. 2013 Nov;5(4):245-255.
29. Muhlemann, J. K., et al. Developmental changes in the metabolic network of snapdragon flowers. *PLoS One*. 2012;7(7):e40381.
30. Liao P, Hemmerlin A, Bach TJ, Chye ML. The potential of the mevalonate pathway for enhanced isoprenoid production. *Biotechnol Adv*. 2016 Sep-Oct;34(5):697-713.
31. Yao RY, Zou YF, Chen XF. Traditional Use, Pharmacology, Toxicology, and Quality Control of Species in Genus *Bupleurum* L. *Chin Herb Med*. 2013 Nov;5(4):245-255.
32. Muhlemann, J. K., et al. Developmental changes in the metabolic network of snapdragon flowers. *PLoS One*. 2012;7(7):e40381.
33. Liao P, Hemmerlin A, Bach TJ, Chye ML. The potential of the mevalonate pathway for enhanced isoprenoid production. *Biotechnol Adv*. 2016 Sep-Oct;34(5):697-713.

Tables & Figure

Table 1 and figure 3 & 5 are available in the Supplementary Files section.

Figures

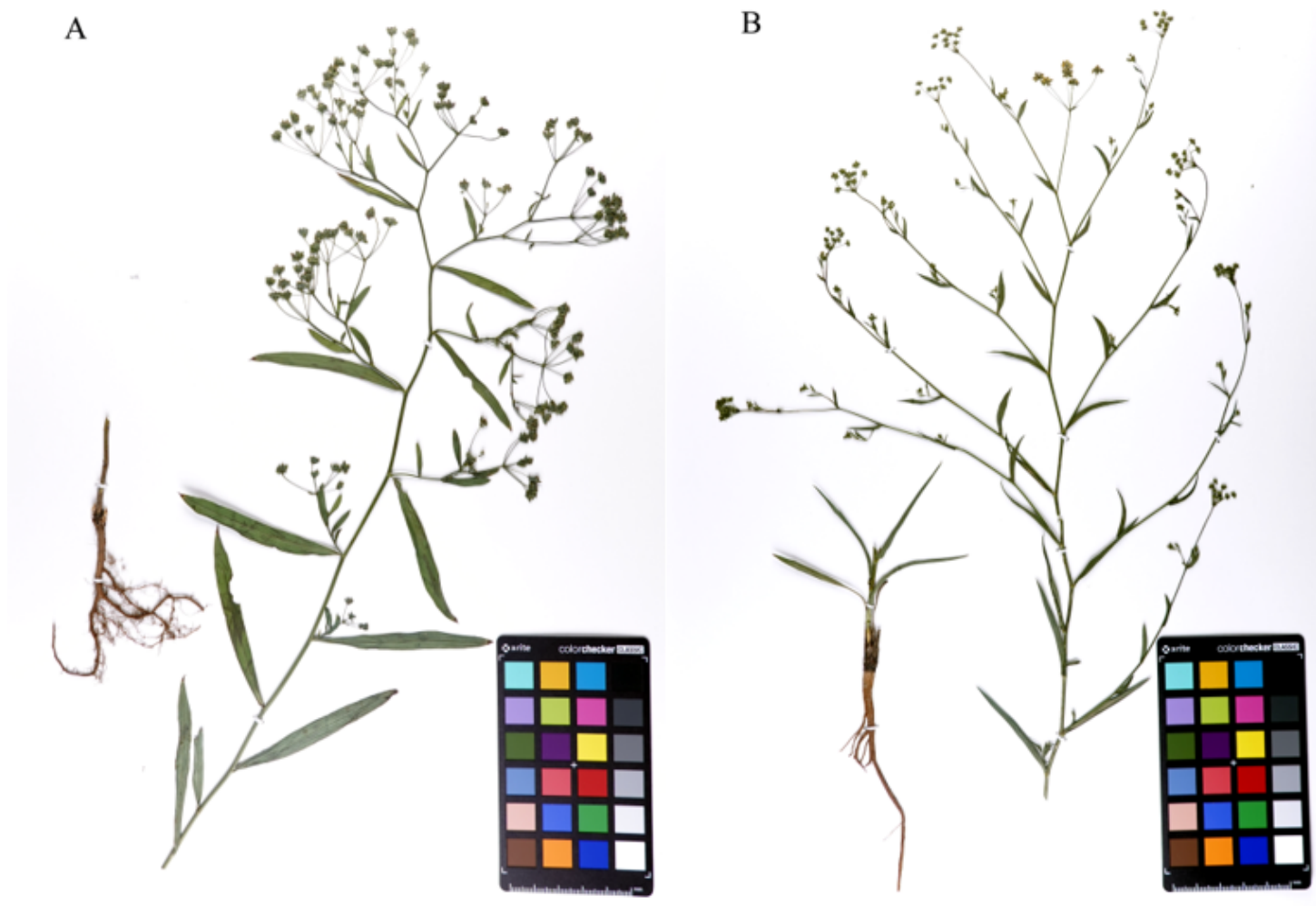


Figure 2

The specimen of mature *Bupleurum chinense* DC.(A) and *Bupleurum scorzonerifolium* Willd.(B).

See Supplementary Files for Figure 3

Figure 3

Overview of annotated metabolites

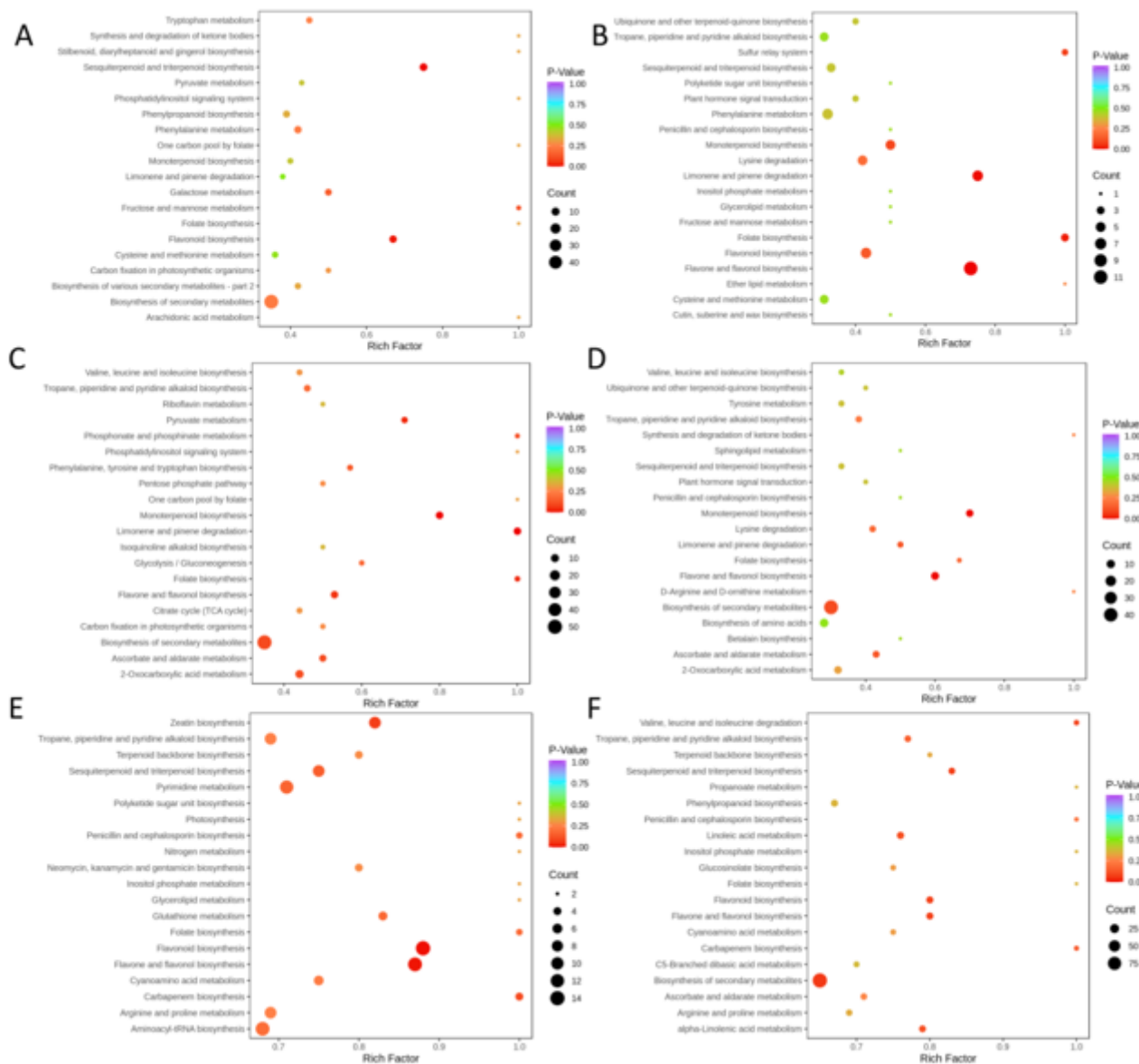


Figure 7

Differential metabolite pathway bubble map. (A) BcR and BsR. (B) BcS and BsS. (C) BcL and BsL. (D) BcF and BsF. (E) BcR and BcF. (F) BsR and BsF.

Supplementary Files

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- [Table1Figure3andFigure5Dincluding3sheets.xlsx](#)