

Assessment of cropping patterns of *Atractylodes lancea* by mass spectrum metabolomics

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Abstract

Background: *Atractylodes lancea* possesses immense potential for application in pharmaceuticals, food and health products, cosmetics, and agricultural production. However, due to the depletion of natural resources, researchers are investigating high-yield and efficient cropping models for *Atractylodes*. Until now, the differences and changes of chemical constituents in *Atractylodes* under different cropping patterns and growth years have been very limited, so further exploration and research are needed.

Materials and methods: Raw materials for *Atractylodes* are supplied by Maoshan Traditional Chinese Medicine Planting Company, a laboratory cooperative planting site. The standard product is provided by China Institute of Food and Drug Control. The samples and standards were dissolved in methanol for analysis during October-December 2022. Tests were performed by ultra-high performance liquid chromatography-mass spectrometry (UPLC-Q/TOF-MS) and gas chromatography-mass spectrometry (GC-MS) for quantitative and metabolomic analysis. Stoichiometric analysis and metabolic pathway analysis were conducted based on mass spectrometry.

Results: After conducting content tests, it was discovered that the intercropped biennial *Atractylodes* contained the required 3% or more atractylodin. After testing, *Atractylodes lancea* samples did not contain phorate pesticides. On this basis, 411 volatile metabolites were extracted and identified, while 111 secondary metabolites were identified. 36 volatile metabolites and 26 secondary metabolites showed significant differences between the two cropping patterns and different growth years. Through metabolic pathway enrichment analysis, it was found that volatile metabolites had significant differences in the biosynthesis of unsaturated fatty acids pathway.

Conclusions: The biennial intercropping approach, yielded *Atractylodes* extracts with content levels that were 1.8, 1.9, and 2.3 times higher than those derived from biennial monocrop, annual intercrop, and annual monocrop, respectively. It was also found that secondary metabolites more accurately reflected the distinctions brought about by the different cropping patterns and growth periods in *Atractylodes*. Hence, this experiment holds significant value as a reference for developing environmentally sustainable agricultural practices for growing this herb. It can also be used to establish an authentic production area model and for the evaluation of herb quality.

Introduction

Atractylodes lancea (Thunb.) DC. (*A. Lancea*) is a perennial herbaceous plant of the *Asteraceae* family. The rhizome of this plant is a primary source of *Atractylodis* Rhizoma, a renowned Chinese medicine that has been in clinical use for several millennia. It was first mentioned in the *Shen Nong Ben Cao Jing* (Han Dynasties), [1] *Atractylodes* is native to various regions of China, and among them, the *Atractylodes* from the Maoshan area in Jiangsu Province stands out for its superior quality, which eliminates dampness, invigorates the spleen, and dispels flatulence [2], which is used in the treatment of rheumatic diseases, digestive disorders, night blindness and influenza. [3] It is commonly utilized in the treatment of

gastrointestinal issues. *Atractylodes* boasts a rich supply of volatile components, specifically sesquiterpene and polyacetylene components.[4, 5] These components play an important role in pharmacodynamics. Among these, the main active ingredients are β -Eudesmol, Atractylodin, Atractylenolide I and Atractylone, which have been extensively studied and shown to have pharmacological effects such as improving gastrointestinal function, anti-tumour, immunomodulation, neuroprotection and anti-oxidation.[6, 7] For instance, *A. Lancea* can prevent diet-induced obesity in mice and mechanism of action involves increasing the metabolic rate in skeletal muscle and brown adipose tissues.[8, 9] Inulin-type polysaccharides, which are abundant in *A. Lancea*, have the physiological functions of water-soluble dietary fibre and bioactive precursors.[10, 11] Atractylone ameliorates mast cell-mediated allergic reactions by inhibiting mast cell degranulation or stabilizing mast cells, and 40 mg kg⁻¹ atractylone reduces influenza A virus-induced lung injury[12–14].

With the increasing demand for *A. Lancea* in the pharmaceutical, health products, animal feed and cosmetics industries, wild *A. Lancea* resources have been gradually reduced or even depleted.[15] At present, the Maoshan producing area, where high-quality *A. Lancea* is produced, has no personnel specializing in wild *A. Lancea* mining, and the output of wild *A. Lancea* has almost disappeared. To protect *A. Lancea*'s resources, we need to pay more attention to the cropping and cropping of *A. Lancea* to ensure its needs in different fields. To safeguard the preservation of natural resources while catering to the escalating demand for *A. Lancea*, extensive artificial cultivation is now underway in Hubei, Jiangsu, and Anhui provinces. This initiative aims to bridge the demand-supply gap while preserving the ecological balance. However, changes in geographical and climatic conditions, differences in artificial cultivation techniques and genetic resources lead to differences in the composition and volatile contents of *A. Lancea*. Moreover, long-term single artificial cultivation leads to the deterioration of the soil environment and a decline in rhizome quality and yield, which affects economic benefits and pharmacological activities. The combination of forest and medicinal plants can give full play to the reciprocal effect of different plants, to promote the healthy growth of understory medicinal plants. It is an ideal planting method with significant advantages of quality preservation and yield increase. In the Maoshan area, forest medicine has been adopted to improve the quality and yield of *A. Lancea*. However, the qualitative and quantitative analysis of the effective components and the differences of metabolites of *A. Lancea* of the same germplasm under different planting patterns are rarely reported at home and abroad. Therefore, this area still needs further study.

Based on this, this paper adopts an *A. Lancea-Evodia rutaecarpa* forest medicinal intercropping system, combining the characteristics of different *A. Lancea* and *Evodia rutaecarpa* growth environments, in order to improve the overall performance.[16] *A. Lancea* grows in scrub and forest grasses and is a rare companion plant. The active ingredients of *Evodia rutaecarpa* have biological control of plant pathogens such as aphids, the most important pest in *A. Lancea*, the indole quinazoline alkaloids and their derivatives in *Evodia rutaecarpa* at 50 $\mu\text{g mL}^{-1}$ resulted in over 250% mortality of aphids, and the limonin were also zootoxic to aphids.[9, 17–19] It can also limit the 'bottom-up' colonisation and growth of other pests such as aphids that feed on *A. Lancea* by physically preventing predation by the host.[20] Forest

medicine intercropping systems, which also mask host plant odours and allow pests to find their hosts. [16] The *A. Lancea-Evodia rutaecarpa* intercropping system used in this study, in which the unique aromatic odour of *Evodia rutaecarpa* effectively masks the odour emitted by *A. Lancea*, thus preventing pest infestation. The use of this system greatly reduces the number of pesticides required during the cultivation and development of *Atractylodes* and is in line with the concept of green development.

Based on the above, different cropping patterns and growing times of *A. Lancea* were selected for observation and comparison in this study, including annual monocrop, annual intercrop, biennial monocrop and biennial intercrop. The samples were analysed by (UPLC-Q/TOF-MS) and (GC-MS). The content of *A. Lancea* was determined and pesticide residues were monitored according to the standards of the Pharmacopoeia of the People's Republic of China (2020 edition).[21, 22] In addition, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were used to analyse the differences in the chemical composition of *A. Lancea* under different cropping patterns and the potential chemical markers for distinguishing between different cropping patterns. The aim of this paper is to study the differences in the volatile oils of *A. Lancea* under different cropping patterns in the Maoshan region, in order to improve the quality of *A. Lancea*, guide breeders and enterprises to carry out reasonable and standardized breeding and cultivation work and provide scientific guidance for industrial development by selecting a highly efficient and effective green industrial cropping patterns to make up for the shortage of wild resources. At the same time, the results of this study will provide an important reference for the revision and improvement of quality standards of *A. Lancea*.

Materials and methods

Plant materials

After selection, we collected *A. Lancea* samples at the Maoshan cultivation site in Jurong, Jiangsu Province (31.89 °N,118.88 °E). To ensure the accuracy and reliability of the experimental results, we chose two cropping patterns and two growth times, with three biological replicates in each case. For the intercropping pattern, we chose the *A. Lancea-Evodia rutaecarpa* intercropping mechanism. These samples were identified as *A. Lancea* compositae by Yuan Changqi, a researcher at the Institute of Botany, Chinese Academy of Sciences, Jiangsu Province. These samples are currently stored in the Natural Products Laboratory at the Institute of Botany, Chinese Academy of Sciences, Jiangsu Province.

Sample collection

Preparation of reference solution: Select an appropriate amount of Atractylodin standard, weigh accurately and add to chromatographic grade methanol in order to make a solution containing 20 micrograms per 1 ml. Sample preparation: Take 0.2 g of this product powder (pass through a No. 3 sieve) and add precisely 50 ml of chromatographic grade methanol into a conical flask with a stopper. Sonication (power 250 W, frequency 40 kHz) was carried out for 1 hour, allowed to cool and then accurately weighed again. The loss of weight was made up with chromatographic grade methanol, shaken well and filtered, and the filtrate of the make-up was taken to obtain the prepared test solution.

Instrumental optimization for GC-MS analysis

Column: Agilent J&W DB-5 ms UI, 30 m × 180 μm × 0.18 μm (part No. 121-5522UI), sample size: 1 μL, inlet mode: shunt, inlet temperature: 270 °C, shunt flow rate: 10 mL min⁻¹, shunt ratio: 10:1, carrier gas: helium, column flow rate: 1.0 mL min⁻¹, column temperature chamber heating procedure: 0 min, 60 °C for 1 min; 10 °C min⁻¹, 180 °C for 1 min; Keep at 20 °C min⁻¹ and 280 °C for 15 min. Ion source: EI, range: m/z 20–550, ion source temperature: 230 °C, quadrupole temperature: 150 °C.

Instrumental optimization for UHPLC-MS/MS analysis

The determination was performed on an InfinityLab Poroshell 120 EC-C18 column, 3.0 × 100 mm, 2.7 μm (P.N. 695975-302), with the column temperature at 40 °C, mobile phase: methanol-water (79:21), sample size: 10 μL (for determination of atractylodin), and flow rate: 0.5 mL min⁻¹, detection wavelength: 340 nm. ESI ion source mass spectrometry detector, positive ion mode (100–1000 m/z); Nebulizer pressure 50 psi; The drying gas flow rate is 10 mL min⁻¹; The drying gas temp is 350 °C; The capillary voltage is 4000 V; Fragmentor voltage is 140 V. The MS/MS collision energy is 15 V.

Data analysis

Qualitative and quantitative analysis of components

We utilized Agilent's MassHunter acquisition software to extract the original data files of GC-MS and UPLC-Q/TOF-MS, obtaining the mass/charge ratio (m/z), retention time (Rt), and peak area of characteristic peaks. We then performed a statistical analysis of the data. To improve the accuracy of our analysis, we implemented several procedures to prepare and organize the data, including determining the retention index, filtering individual peaks, preserving peak area data with a single null group < 50% or all null groups < 50%, and normalizing using data from internal standard normalization. Additionally, we consulted multiple reputable sources, such as CNKI, Geen Medical, Sci Finder, and the Shanghai Institute of Organic Chemistry to establish a comprehensive database of *A. Lancea* related components. This database included information such as compound names, chemical formulas, relative molecular weights, and structural formulas. Finally, we combined a qualitative and quantitative analysis workflow with the NIST database and our self-built *A. Lancea* compound database to conduct qualitative and quantitative analysis of GC-MS and UPLC-Q/TOF-MS data.

Statistical analysis

We used MassHunter Profinder software and Unknowns analysis software to extract molecular signatures by analysing the mass spectrometry data obtained. At the same time, we aligned the data accurately and set up reproduction conditions to rediscover the ions. The processed data were subjected to unsupervised principal component analysis (PCA) and supervised partial least squares analysis (PLS-DA) using SIMCA 14.1 for all pre-processed data. Using Agilent Mass Profiler Professional (MPP) software in combination with PLS-DA,[23] we obtained variable weight values (VIP > 1.2) or T-test ($P < 0.05$) and Fold change (FC) ≥ 2 to look for potential chemical differences as well as metabolite changes,

stratified clustering of metabolites, and presented as Heat map representation. The effects of different growth states on metabolic pathways and interactions of *A. Lancea* were studied by KEGG pathway (<https://www.kegg.jp>) enrichment analysis.

Results

Characteristics of *A. Lancea* in intercropping and monocropping modes

For different cropping patterns, fresh cross-sections of *A. Lancea* showed different characteristics: biennial *A. Lancea* had a larger, smoother surface; but annual *A. Lancea* was small overall. Four samples of *A. Lancea* were cut across and a yellow-white texture was observed, as shown in Fig. 1C. The biennial cross-section was longer, around 2 cm in diameter, and the annual cross-section was shorter, around 1 cm in diameter. Compared to the monocrops, the intercrops had closely arranged fibre bundles and a more dense distribution of oil spots. It is worth mentioning that *A. Lancea* is rich in oil, starch and mucilage, which can enhance the medicinal value of *A. Lancea*.

Identifying metabolites of *Atractylodes*

After integrating and calibrating the peaks using MassHunter and Unknown Analysis software, we successfully located and identified 411 compounds detected by GC-MS and 111 compounds detected by UPLC-Q/TOF-MS. We utilised NIST libraries, public data, self-built databases and laboratory standards to support this process and provided accurate supporting information for each compound, including number, name, molecular formula, molecular weight, retention time, CAS (Chemical Abstracts Service) or PubChem CID and VIP value. Detailed data have been collated in Table S1 and Table S2.

Analysis of the content of atractylodin

The test and control samples were prepared according to the method in item 2.2 and determined using the chromatographic conditions under items 2.3/2.4. And the concentration of the standard was used to calculate the content of atractylodin in the sample. The specific calculation formula was: the peak area of the standard divided by the peak area of the sample was equal to the ratio of the concentration of the standard to the concentration of the sample. The final results are detailed in Table 1 and Table 2.

Table 1
Content of atractylodin detected by UPLC-Q/TOF-MS

Sample	RT (min)	Final Conc. ($\mu\text{g mL}^{-1}$)	The content of atractylin	The pharmacopoeia states not less than 0.30%
Atractylodin Standard	7.47	19.9	99.5%	
Annual monocrop	7.36	20.6	0.52%	Compliant
Annual monocrop	7.39	9.8	0.24%	Non-compliant
Annual monocrop	7.22	10.8	0.27%	Non-compliant
Annual intercrop	7.10	17.1	0.43%	Compliant
Annual intercrop	7.08	20.8	0.52%	Compliant
Annual intercrop	7.23	10.9	0.27%	Non-compliant
Biennial monocrop	7.32	18.6	0.47%	Compliant
Biennial monocrop	7.35	13.9	0.35%	Compliant
Biennial monocrop	7.09	19.8	0.49%	Compliant
Biennial intercrop	7.09	44.1	1.10%	Compliant
Biennial intercrop	7.08	23.3	0.58%	Compliant
Biennial intercrop	7.19	26.8	0.67%	Compliant

Table 2
Content of atractylodin detected by GC-MS

Sample	RT (min)	Final Conc. ($\mu\text{g mL}^{-1}$)	The content of atractylin	The pharmacopoeia states not less than 0.30%
Atractylodin Standard	15.82	19.9	99.5%	
Annual monocrop	15.79	3.9	0.10%	Non-compliant
Annual monocrop	15.81	4.2	0.11%	Non-compliant
Annual monocrop	15.81	6.4	0.16%	Non-compliant
Annual intercrop	15.82	9.4	0.24%	Non-compliant
Annual intercrop	15.80	25.4	0.44%	compliant
Annual intercrop	15.84	12.8	0.32%	Compliant
Biennial monocrop	15.79	9.1	0.23%	Non-compliant
Biennial monocrop	15.82	10.2	0.26%	Non-compliant
Biennial monocrop	15.80	11.1	0.28%	Non-compliant
Biennial intercrop	15.80	11.1	0.28%	Non-compliant
Biennial intercrop	15.79	15.1	0.38%	Compliant
Biennial intercrop	15.79	14.7	0.37%	Compliant

Analysis of pesticide residues

Phorate has a high insecticidal effect and can be widely used for the control of aphids, termites and other pests and mites, with characteristics such as persistence and rapidity. However, the highly toxic nature of this pesticide poses serious hazards to the environment and human beings. In this experiment, we successfully obtained the ion flow map of *A. Lancea* by GC-MS and attempted to extract the molecular ion peaks M^{+} 260.1 and fragment ions 75 and 97 for phorate. We also attempted to extract the molecular ion peaks of the structural analogues M^{+} 244, 276 and 292 for phorate but failed to find the relevant structural peaks, as shown in Fig. 2. After careful examination, we were unable to detect the presence of this pesticide component in the extract of *A. Lancea*.

Principal component analysis

The principal component analysis is a commonly used data analysis technique to extract key information from a large number of variables and measure the total variance of the data with fewer variables, thereby simplifying the data set and reducing the complexity of the data analysis. The experimental results are shown in Fig. 3. The GC-MS data results are plotted in A, with insignificant intra-group differences between the four cropping patterns. The UPLC-Q/TOF-MS data results are plotted in B. In the positive ion model, we found relatively significant differences between *A. Lancea* in different growth environments, and these results were largely within the confidence intervals. The chemistry of annual *A. Lancea* was significantly different from that of biennial *A. Lancea*, while the similarity between the biennial *A. Lancea* groups was relatively high. We also found that the GC-MS assay results did not present as significant an effect as the UPLC-Q/TOF-MS, suggesting that the secondary metabolites of *A. Lancea* are more useful in discerning the differences between different cropping patterns of *A. Lancea*. To exclude the effect of within-group error, we excluded random error in the between-group difference analysis and further used partial least squares discriminant analysis to gain insight into the differences between different groups of *A. Lancea*.

Partial least squares discriminant analysis

In PLS-DA, we use principal component analysis to downscale high-dimensional data to lower dimensions and determine an optimal hyperplane for classification by minimising the gap between inter-class variance and intra-class variance. With appropriate data pre-processing and model optimisation, PLS-DA can improve the accuracy and reliability of the classification. We have validated the effectiveness of GC-MS and UPLC-Q/TOF-MS techniques for detecting the quality of compounds using a PLS-DA-based approach. In Fig. 4, we show the score plots of the first two potential variables obtained from PLS-DA, presenting the results of the sample distributions. Panel A shows the results of the GC-MS modelling analysis with a cumulative explanatory power parameter $R^2Y(\text{cum})$ of 0.991 and a predictive power parameter $Q^2(\text{cum})$ of 0.557; panel B shows the results of the UPLC-Q/TOF-MS modelling analysis with a cumulative explanatory power parameter $R^2Y(\text{cum})$ was 0.986 and the predictive power parameter $Q^2(\text{cum})$ was 0.664. These two indicators show that the model was well-fitted and predicted. It can be seen from the graphs that the secondary metabolites of *A. Lancea* are more conducive to the differentiation and screening of differential metabolites. From the GC-MS plot, the differences between the biennial *A. Lancea* samples were small and may not be well differentiated. In contrast, the UPLC-Q/TOF-MS plots show that there are significant differences between the groups, which are more conducive to differential analysis for different cropping patterns of *A. Lancea*, which also corroborates the conclusions of the PCA analysis.

Differential metabolite analysis

The authors further analysed the differences in metabolites accumulated between *A. Lancea* samples under different cropping patterns. Firstly, 66 primary metabolites and 483 secondary metabolites were screened by setting parameters of $P\text{-value} < 0.05$, retaining entities that appear in at least 80% and $\text{FC} \geq 2$. In addition, one, three, one and two specific secondary metabolites were found when four cropping patterns, annual monocrop, annual intercrop, biennial monocrop and biennial intercrop, were studied,

respectively, the Venn diagram is shown in Fig. 5. For the secondary metabolites, volcano plots of the differential metabolites were analysed in Fig. 6, where the annual intercrop was used as a control group with a Y -coordinate of $-\log_{10}(p\text{-value})$ and an X -coordinate of $|\log_2(\text{fold change})|$. Each point represents a metabolite, with red indicating metabolites with significantly up-regulated expression, blue indicating metabolites with significantly down-regulated expression, and other colours representing metabolites with no significant differences. From the results of the shared metabolites, we conclude that differences in cropping patterns had little effect on the variety of metabolites in *A. Lancea*. As the 12 batches of *A. Lancea* tested were all of the same germplasm, the results obtained are acceptable and consistent with reality.

Cluster analysis of different metabolites

We investigated the discrepancy data of UPLC-Q/TOF-MS and GC-MS samples using the PLS-DA analytical method. In performing the variance analysis, we screened compounds with a coefficient of variation $VIP > 1.2$ for analysis. Correlation analysis is an effective method to identify metabolic similarity among *A. Lancea* in different growing environments. In our study, Pearson analysis was used to identify linear relationships between 36 volatile metabolites and 26 secondary differentially expressed metabolites, and the results were shown in the correlation matrix as shown in Fig. 7. The clustering analysis allowed us to clearly see the grouping pattern of the *A. Lancea* samples under different cropping patterns. Figures 8 and 9 reveal that 36 different metabolites were detected by GC-MS and 26 by UPLC-Q/TOF-MS, respectively. The results from both assays clearly indicated that remarkable variations exist in the levels of metabolites in *A. Lancea* across the four cropping patterns. The unique metabolic profiles observed between different groups signify the potential of *A. Lancea* as a promising bioresource with diverse applications. In a noteworthy point, biological replicates from the same treatments were clustered together, indicating a high degree of homogeneity between them. This therefore also provides a strong assurance of the reliability of the data.

Pathway analysis

KEGG is a database containing extensive information on metabolite pathways and interactions. In this study, all differential metabolites are listed and annotated in the KEGG pathway database. For volatile compounds, 37 out of 411 differential metabolites were successfully annotated in the KEGG pathway database, and 25 out of 111 secondary metabolic differential compounds were successfully annotated in the KEGG pathway database. After enrichment analysis, the pathways that significantly affected the synthesis of multiple metabolites were shown in Fig. 10a, 10b. Each bubble in the visualization represents a metabolic pathway. The size and position of the bubble on the x -axis indicate the degree of influence the pathway has in the topological analysis. The colour of the bubble illustrates the p -value of the analysis and shows that a lower p -value indicates a higher degree of significance for the enrichment. The rich factor is the percentage of differential metabolites in the detected metabolites within the pathway. By evaluating P -values and enrichment factors, we found that the Biosynthesis of unsaturated fatty acids (hsa01040, $p < 0.001$) was the most significantly different pathway among the four *A. Lancea* growth environments.

Discussion

A. Lancea is an important medicinal herb that can be divided into Southern *A. Lancea* (a.k.a.: Mao *A. Lancea*) and Northern *A. Lancea* according to its base source. According to ancient texts, “*Atractylodes* is produced in the Mao Mountains and is best when it has thin stalks with black skin and red dots inside”. It is also characterised by its distinctive “vermillion dots” and “white frost”, which make it very easy to identify. However, due to the impact of mining, afforestation and indiscriminate mining and digging in the hilly areas of the Maoshan Mountains, wild resources have been destroyed and the production of *A. Lancea* has plummeted, with wild resources becoming scarce and prices remaining high. This problem has attracted the attention of Hubei, Jiangsu and Anhui, and some artificial cultivation bases have been established and standardised cropping methods have been adopted. Currently, on the market, about 70% of *A. Lancea* comes from cultivation in Ying Shan, Hubei. As technology continues to advance, the application areas of *Atractylodes* are expanding and the market demand is bound to grow. Therefore, in the development strategy of *A. Lancea*, we need to pay attention not only to the development of production areas with concentrated resources and large reserves but also to the protection of resources in the authentic production areas, the development of suitable cropping patterns and the development of authentic varieties, both to protect and to make use of, in order to allow *A. Lancea* to be better developed and utilised.

In this study, an efficient method was developed to trace cropping *A. Lancea* patterns by UPLC-Q/TOF-MS, GC-MS and chemical pattern recognition analysis. The UPLC-Q/TOF-MS fingerprint analysis showed that *A. Lancea* was similar across cropping patterns. The differences were also significant. A combination of PCA and cluster analysis of the relative contents of the characteristic compounds revealed that the main components of the volatile oils of *A. Lancea* differed between the different cropping patterns. In addition, the relative contents of samples from different cropping patterns also differed significantly. Analysis of variance combined with PLS-DA analysis revealed four compounds with significant differences in the GC-MS results (VIP > 1.7), namely dihydroxyacetone, atractylenolide I, atractylenol and hinesol. In addition, three compounds with significant differences in the UPLC-Q/TOF-MS (VIP > 1.6) were also found, namely alpienoside, reptoside and dihydrophloroglucinol. These compounds may be important chemical markers to distinguish between different cropping patterns. Furthermore, when compared to alternative synthesis pathways, it was observed that there were statistically significant variances in the biosynthesis of unsaturated fatty acids in *A. Lancea* under varying growth conditions.

In the study, various advanced analytical techniques such as UPLC-Q/TOF-MS, GC-MS and chemical pattern recognition were used to comprehensively reveal the effects of different cropping patterns on the chemical composition and appearance of *A. Lancea*. By incorporating metabolites as a highly reliable indicator, we have effectively established a means of tracking cropping patterns with precision and accuracy. This result is of greater reference value for similar studies. The quality and appearance traits of Chinese herbs are closely related to their intrinsic chemical composition, however, the influence of environmental factors may lead to differences. For *A. Lancea*, varietal factors dominate in shaping its chemical composition. In this work, we systematically investigated the effects of different cropping

patterns on the appearance and chemical composition of *A. Lancea* based on the same species. According to the Chinese Pharmacopoeia, the harvesting period for *A. Lancea* should take place in the autumn of two years or in the spring of three years, with three years being generally preferable. However, experiments have shown that intercropping varieties of *A. Lancea* can also be harvested in the autumn of the second year and that their appearance and chemical composition meet the required standards. Shortening the harvesting time not only complies with the regulations but also promotes the development of the herb-growing industry and improves economic efficiency. At the same time, ecological cultivation is developing rapidly. The intercropping model of *A. Lancea-evodia rutaecarpa* used in this paper has demonstrated the advantages of its above-ground component. In the future, comprehensive studies can be conducted to assess the ecological effects of this intercropping method on both the environment and the soil microorganisms within the root zone. This provides an important reference for guiding the breeding techniques of *A. Lancea*, expanding the production of high-quality *A. Lancea*, and solving the problems of scarcity of wild resources and insufficient supply in authentic production areas.

Abbreviation

Atractylodes lancea	A. Lancea
utrl high performance liquid chromatography-mass spectrometry	UPLC-Q/TOF-MS
gas chromatography-mass spectrometry	GC-MS
principal component analysis	PCA
partial least squares discriminant analysis	PLS-DA

Declarations

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Author contribution

Mengdi Wang: Manuscript writing, data processing. Min Yin: Sample testing, article polishing. Xuhong Yong: Data analysis, literature survey. Xiaoxiao Xu: Article polishing, formatting polishing. Shoutao Jing:

Sample collection and processing. Xu Feng: Article polishing, data feasibility analysis. Qizhi Wang: Manuscripts are polished, arranged and financed.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest The authors declare no conflict of interest.

Ethics approval and consent to participate

This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Competing interests

The authors declare that they have no competing interest.

Consent for publication

The authors agreed to the publication of the manuscript in this journal

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Figures

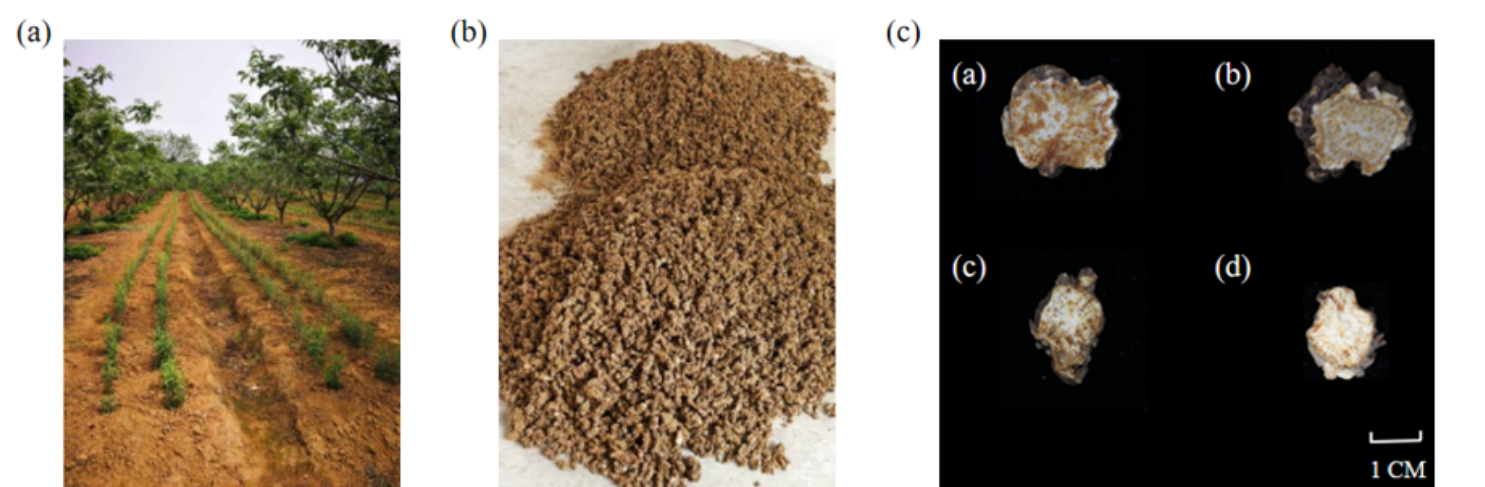


Figure 1

a *Atractylodes-Evodia rutaecarpainter cropping system*. **b** Autumn harvest of *Atractylodes*. **c** Cross-section of dried *Atractylodes*: **a** for biennial intercrop, **b** for biennial monocrop, **c** for annual intercrop, **d** for annual monocrop

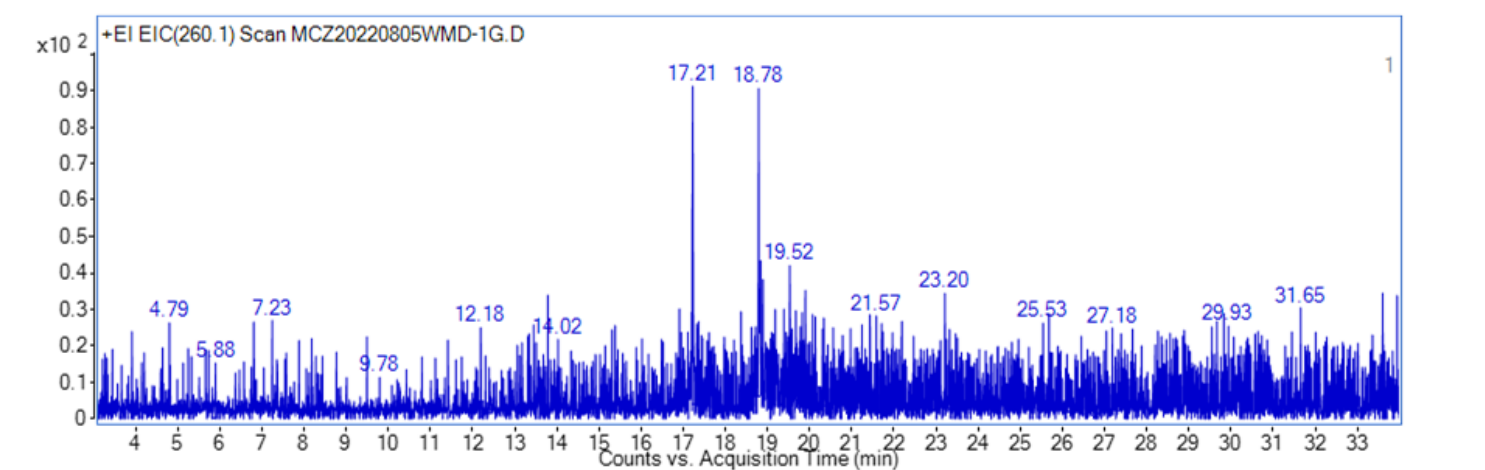


Figure 2

Ion flow diagram of extraction of *Atractylodes lancea* by GC-MS Phorate M⁺ 260.1

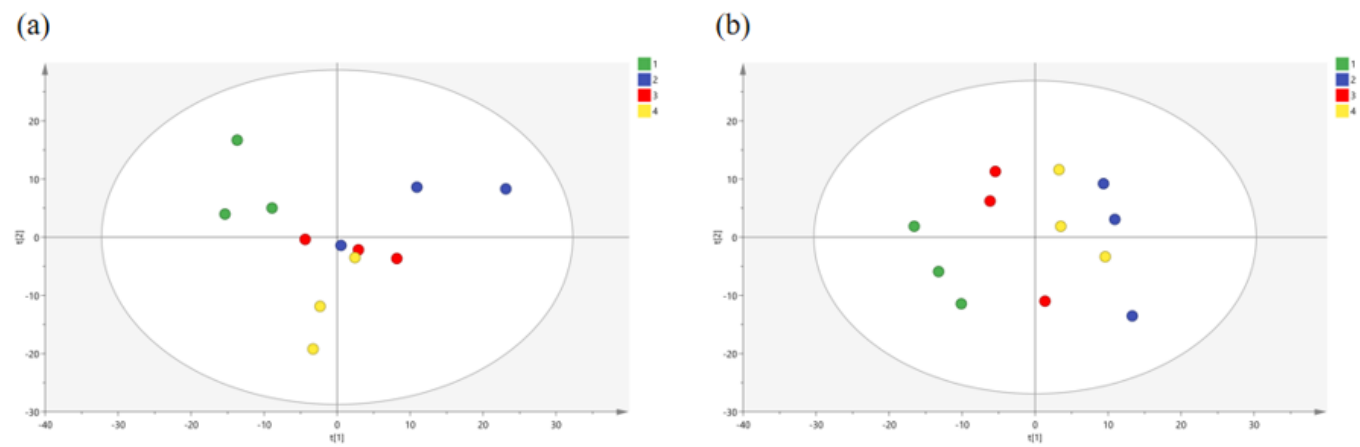


Figure 3

a PCA results based on all compounds by GC-MS. **b**PCA results based on all compounds by UPLC-Q/TOF-MS

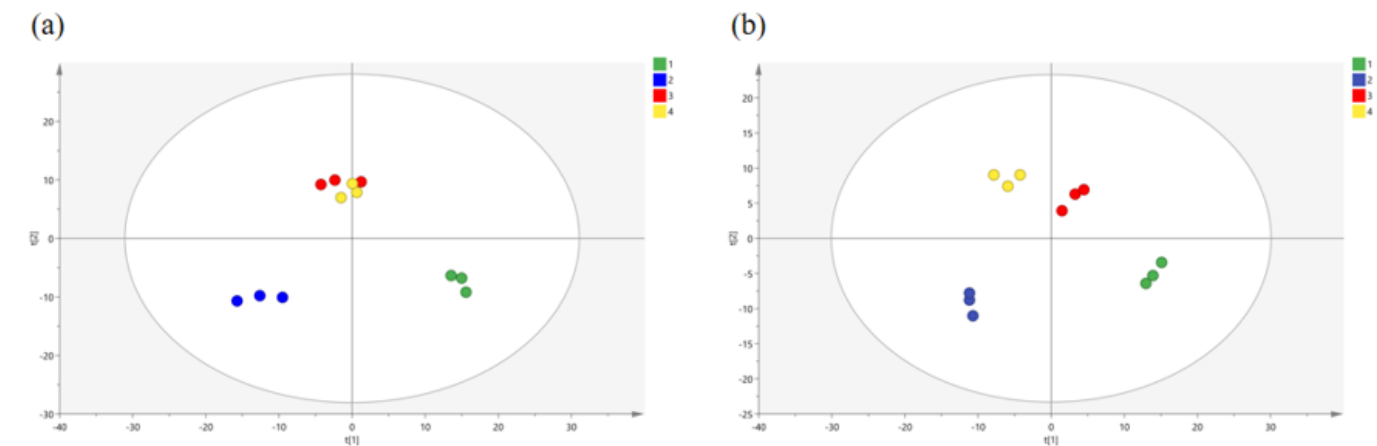


Figure 4

a PLS-DA results based on all compounds by GC-MS. **b** PLS-DA results based on all compounds by UPLC-Q/TOF-MS

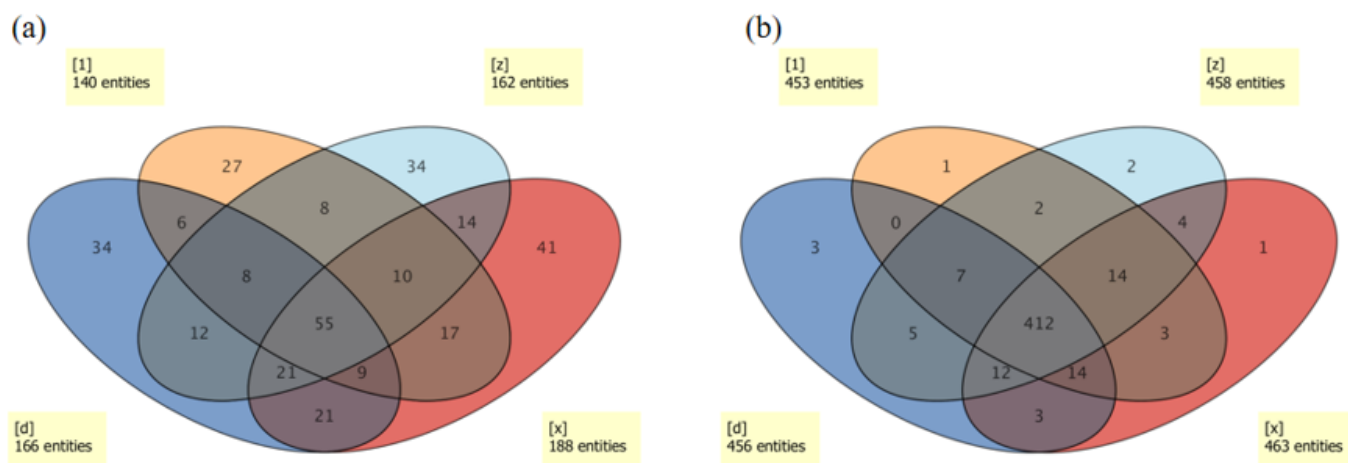


Figure 5

a Venn results based on screened compounds by GC-MS. **b** Venn results based on screened compounds by UPLC-Q/TOF-MS

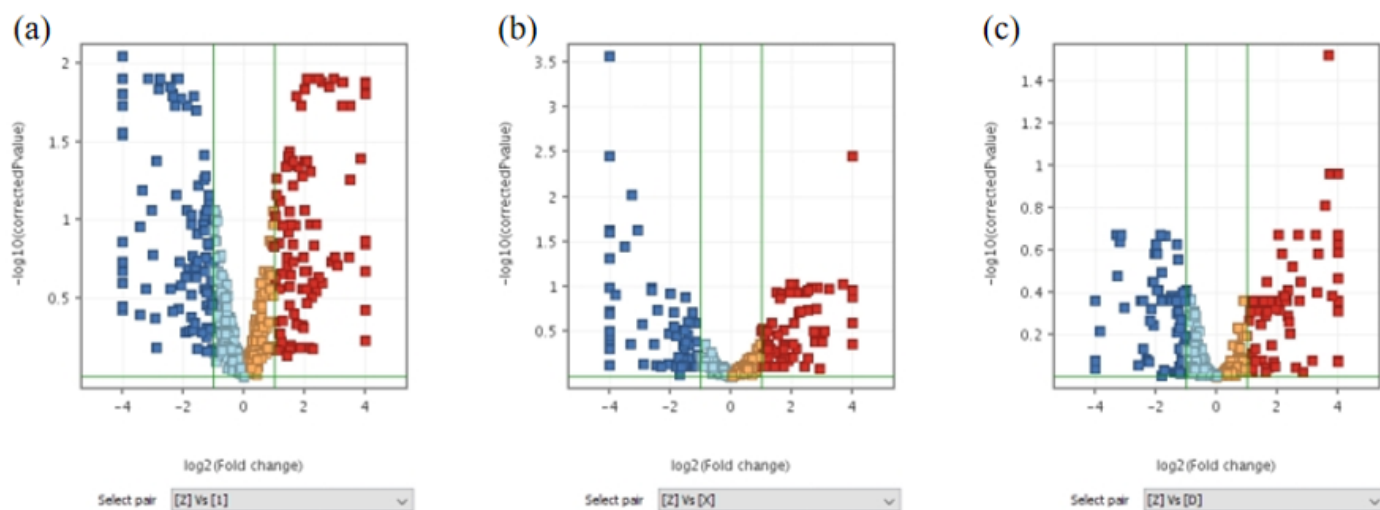


Figure 6

Volcano map of the secondary metabolites of *A. Lancea*. **a** Biennial intercrop vs Annual monocrop. **b** Biennial intercrop vs Biennial monocrop. **c** Biennial intercrop vs Annual intercrop

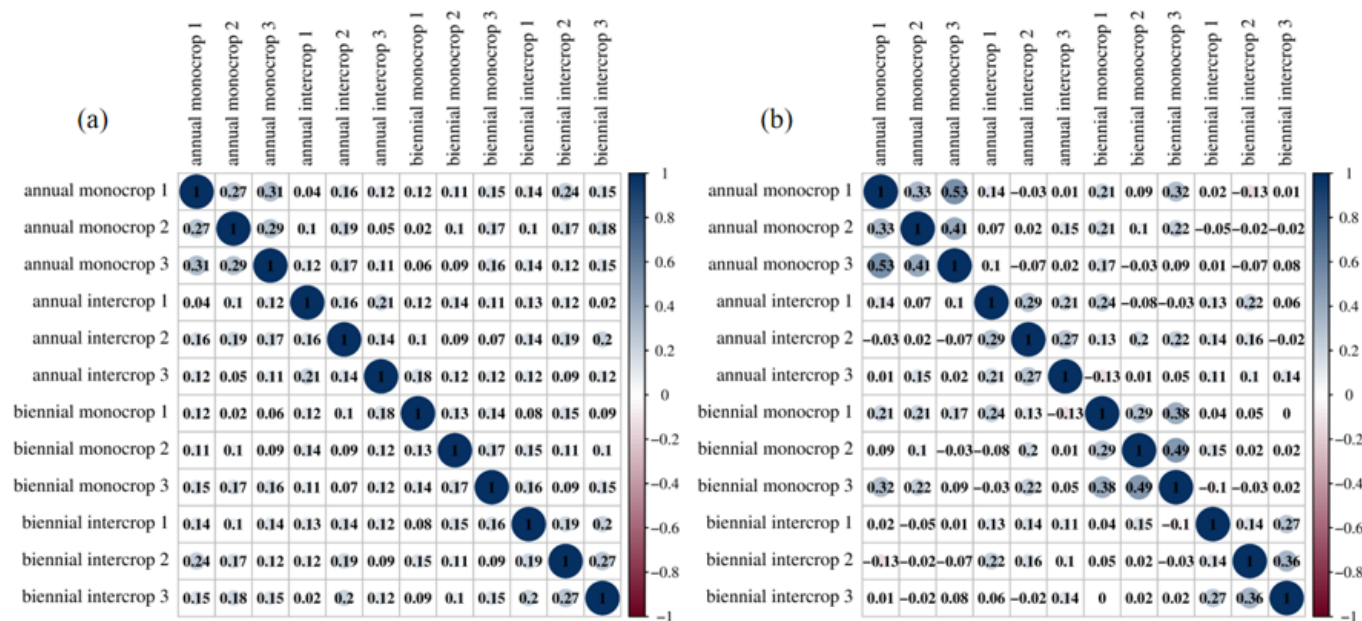


Figure 7

Metabolite-metabolite correlation analysis. Every square represents the Pearson's correlation coefficient for a pair of metabolites. **a** GC-MS results. **b** UPLC-Q/TOF-MS results



Figure 8

GC-MS clustering diagram of differential compounds

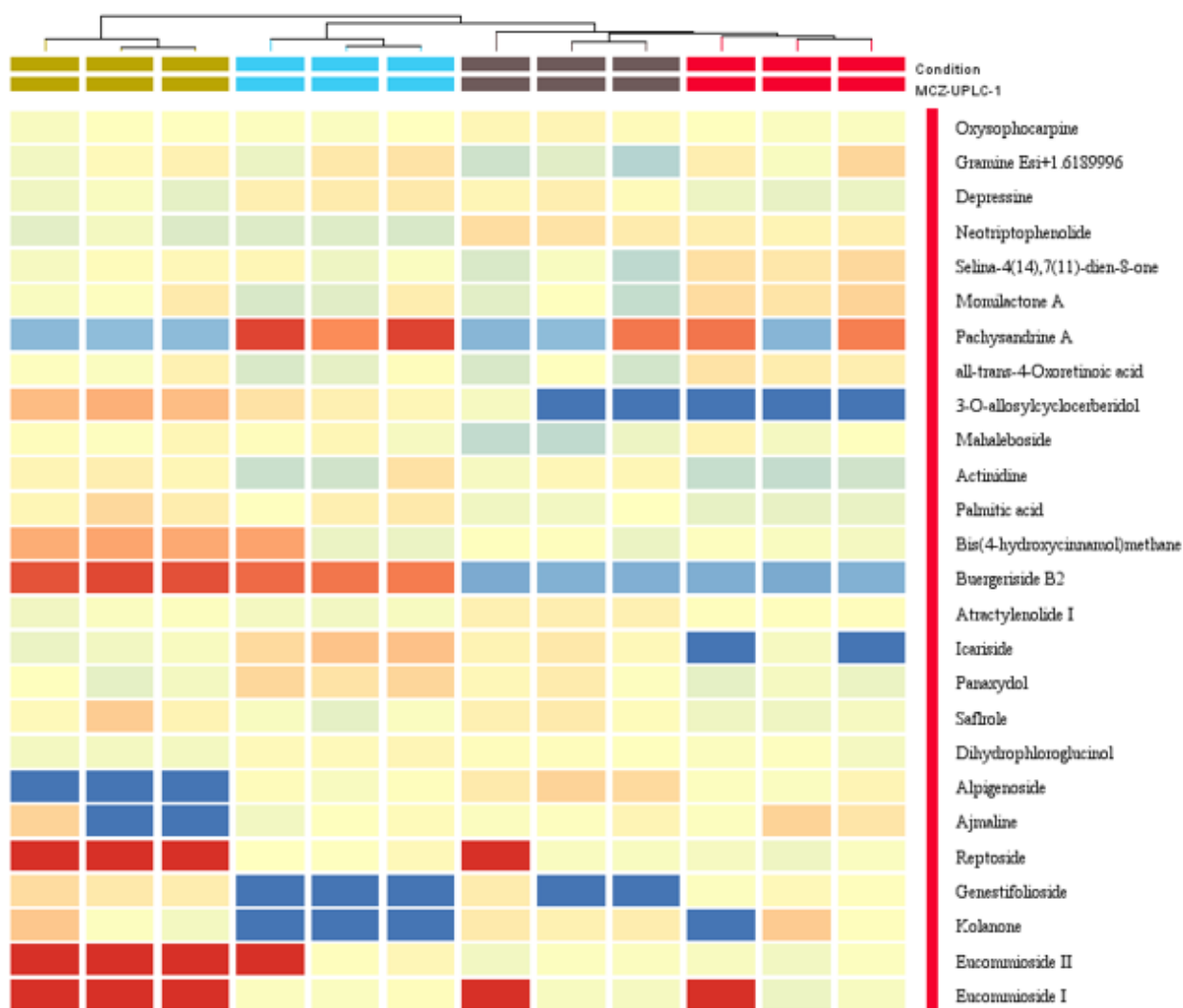


Figure 9

UPLC-Q/TOF-MS clustering diagram of differential compounds (Red indicates annual monocrop; grey indicates annual intercrop; blue indicates biennial monocrop; yellow biennial intercrop)

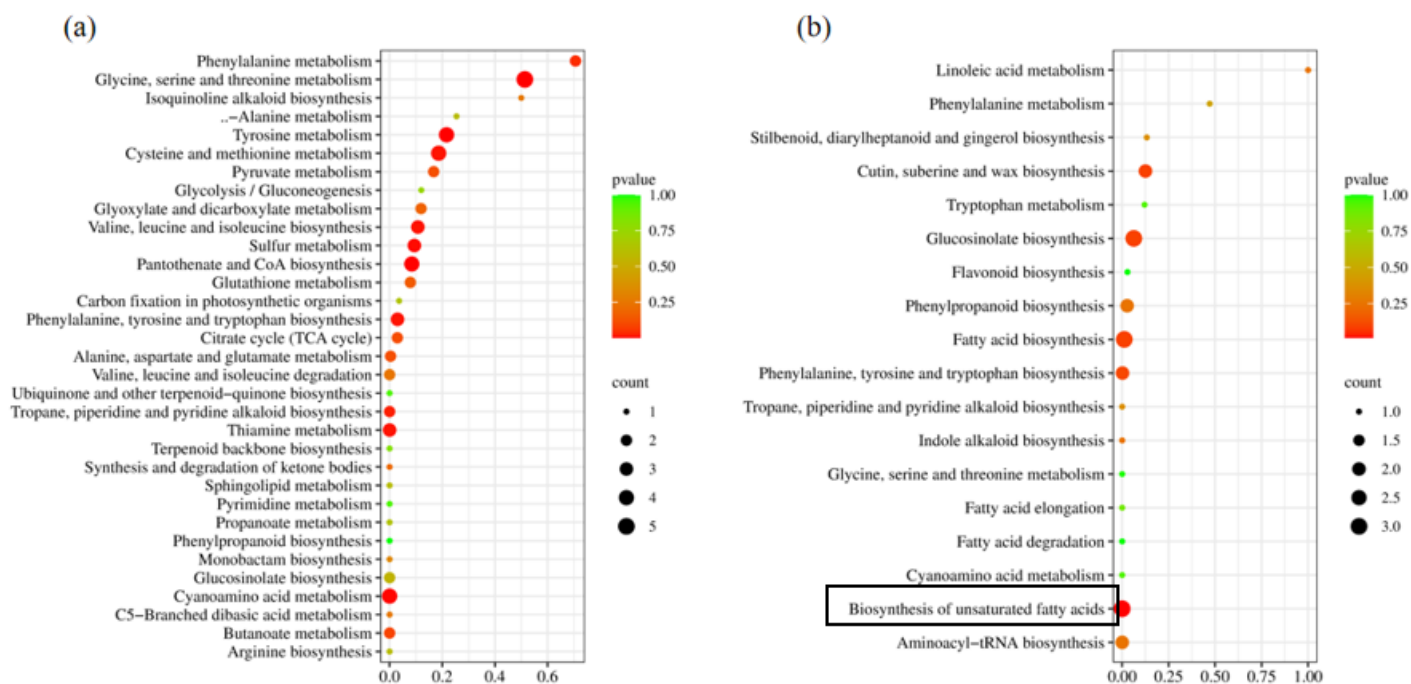


Figure 10

The KEGG pathway enrichment analysis. **a** Results of enrichment analysis based on GC-MS data. **b** Results of enrichment analysis based on UPLC-Q/TOF-MS data

Supplementary Files

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