**Wide Targeted Metabolomics Analysis of the Main Bioactive Ingredients in Serissa japonica (Thunb.) Thunb.**

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**Abstract.** Objective: To study the metabolite composition from Serissa japonica, explore the substances that play a role in this medicine, and lay the foundation for subsequent pharmacological research. Methods: The different metabolites in Serissa japonica (Thunb.) Thunb. were detected by UPLC-MS/MS technology. Results: The findings indicated that a total 719 differential metabolites in Serissa japonica (Thunb.) Thunb., which are divided into 12 categories, including amino acids and derivatives, phenolic acids, nucleotides and derivatives, flavonoids, quinones, lignans and coumarins, tannins, alkaloids, terpenes, organic acids, lipids, and others. The dominant metabolites are flavonoids, phenolic acids, and lipid compounds. Modern pharmacological research has found that Serissa japonica contains glycosides, tannins, phytosterols, a small number of alkaloids, sugars, and fatty acids, which are the main direction of pharmacological research. Conclusion: The composition of the substances was tested by metabolomics in Serissa japonica (Thunb.) Thunb. for the first time, which can lay the foundation for the study of the material basis for the effectiveness of traditional Chinese medicine formulas and subsequent pharmacological studies, and maximize the role of ethnic medicine.

**Keywords:** Serissa japonica; widely targeted metabolomics; metabolites.

1. Introduction

Serissa japonica (Thunb.) Thunb. belongs to the Rubiaceae family, which is mainly distributed in East China, South China, Guizhou, Yunnan and other regions. The whole plant has a bitter taste, slightly sweet, cool in nature, has the effect of dispelling wind and dampness, clearing heat and detoxifying, and can be clinically used for kidney diseases such as rheumatic pain, acute and chronic hepatitis, pyelonephritis, cough, sore throat, swollen and sore gums and other diseases. At present, the research on the chemical components of the plant of the genus is mainly on volatile oils, terpenes, lignin, and quinones [1], and no metabolome analysis has been reported. Therefore, in order to further elucidate the active substance composition of Serissa japonica, this study tested and analyzed the metabolites in Serissa japonica based on a widely targeted metabolomics method by UPLC-MS/MS, in order to provide a theoretical basis for the rational development of its pharmacodynamic material basis, clinical application and resources.

Metabolomics is a method to systematically study all metabolites in living organisms, which can comprehensively understand the composition and change laws of metabolites in living organisms, and provides an important means for in-depth study of metabolic processes in living organisms, and is applied to drug toxicology research, elucidating the mechanism of action of drugs, pharmacodynamics and clinical evaluation. Targeted metabolomics is a mass spectrometry-based metabolomics approach that enables quantitative or semi-quantitative analysis of specific metabolites or metabolic pathways. The advantages of targeted metabolomics are that it can improve the accuracy, sensitivity, and reproducibility of the analysis, as well as reduce the complexity of data processing. With the development of traditional Chinese medicine and modern
medicine, more and more researchers of traditional Chinese medicine use metabolomics technology to study the mechanism of traditional Chinese medicine in the treatment of diseases from the aspects of biochemical reactions, pathophysiological changes, and pharmacological effects of traditional Chinese medicine. After the TCM compound enters the body, it is not only the original components of the TCM that play a pharmacological role, but also the metabolites or active ingredients, as well as the role of the human body in producing new components [2]. Traditional Chinese medicine is the result of the synergistic effect of multiple components, multiple targets, and multiple pathways, and for traditional Chinese medicine compounds, we should focus on the holistic effect of traditional Chinese medicine formulas, rather than focusing on the effects of a single chemical component [3]. In the research of traditional Chinese medicine, metabolomics provides a more reliable research method for the study of multi-component, multi-target, and multi-metabolic pathways, provides strong support for the in-depth study of subsequent metabolites, and also provides more scientific experimental basis for the study of the mechanism of action of traditional Chinese medicine.

2. Experimental Rart

2.1 Subjects of the Study

The Serissa japonica comes from the southeast of Guizhou (26°26’ 46.84" N 107°45’54.17" E), and the medicinal materials have been identified by experts from Kaili Traditional Chinese Medicine Hospital. In this study, fresh and well-growing seedlings were collected, put into a Ziplock bag, labeled, and brought back to the laboratory, cleaned and stored in a -80°C refrigerator for later use. The whole plant was taken, liquid nitrogen was added, ground and crushed in a mortar, mixed evenly, and 5 g samples were weighed and sent to Wuhan Maitwell Biotechnology Co., Ltd. for extensive targeted metabolomics analysis.

2.2 Metabolite Qualitative and Data Analysis

Based on the self-built database MWDB (metware database), the substance is qualitative according to the secondary spectrum information, and the isotope signal, the repeated signal containing K+ ions, Na+ ions, NH4+ ions, and the fragment ions that are other substances with larger molecular weight are removed during the analysis. Screened data were analyzed, and histograms were plotted based on metabolite primary classification and enrichment of metabolite numbers. In order to further display the metabolite data, the primary classification, secondary classification and main enrichment metabolites of metabolites were counted, and the data display table was drawn.

3. Results

3.1 Qualitative and Quantitative Analysis of Metabolites

Mass spectrometry data were processed using the software Analyst 1.6.3, and Fig. 1 shows the total ion flow of the multiplexed QC sample, with the abscissa representing the retention time (Rt) of the metabolite assay and the signal intensity (CPS) of the ions representing the ordinate. On this basis, the mass spectrometry data were compared with the local metabolic database, and the detected metabolites were analyzed qualitatively and quantitatively. Specifically, the mass spectrometry data were analyzed by MultiaQuant software, the chromatographic peaks were integrated and corrected, and the peak area (Area) of each chromatographic peak represented the relative content of metabolites, and finally all chromatographic peak area integration data were exported for storage.
3.2 Metabolite Species Identification

A total of 719 metabolites were identified in the screened data, including 12 types of substances, including amino acids and derivatives (84), phenolic acids (104), nucleotides and their derivatives (47), flavonoids (77), quinones (13), lignans and coumarins (39), tannins (3), alkaloids (43), terpenoids (41), organic acids (51), lipids (133) and others (84). The largest number of enriched metabolites were lipids (133), phenolic acids (104), amino acids and their derivatives (84). According to the primary classification of metabolites and the number of enriched metabolites, a histogram was plotted, and the results are shown in Figure 2.
3.3 Identification of major enrichment metabolites

In order to further display the metabolite data, the secondary classification of various metabolites and the main enriched metabolites were counted, and the results are shown in Figure 3.

![Fig. 3 Relative content of main metabolites](image)

The results showed that the lipids contained 14 glycerides, 1 phosphatidylcholine, 2 sphingolipids, 29 lysophosphatidylcholine, 14 lysophosphatidylethanolamine and 73 free fatty acids, including γ-linolenic acid* (13.34%), α-linolenic acid* (12.60%), 9,10,13-trihydroxy-11-octadecenoic acid (11.14%), lysophosphatidylcholine 18:2 (2n isomer) (7.78%), and lysophosphatidylcholine. The relative content of 16:0 was higher (5.81%). There were 104 kinds of amino acids and their derivatives, among which L-arginine (17%), cycloleucine (11.60%), L-aspartic acid-O-diglucoside (7.05%), L-proline (5.3%) were relatively abundant. Amino acids and their derivatives contained 4 chalcones, 6 dihydroflavonoids, 2 dihydroflavonols, 1 dihydroisoflavone, 19 flavonoids, 33 flavonols, 5 flavonols, 2 isoflavones, and 5 other flavonoids, including 7-methoxy-3-[1-(3-pyridyl)methylene]-4-dihydrochromogenic ketone (59.78%), alfalfa-7-O-glucuronedide (8.82%), 2,4,2’,4’-tetrahydroxy-3’-isopenenylchalcone (8.60%). The relative contents of 1,2,3,7-tetramethoxyxanthone (7.55%) and 5,6,7,5’-tetramethoxy-3’,4’-methylenedioxyflavonoids (4.19%) were relatively high. The organic acids contained 62 substances, among which 2-hydroxyhexadecanoic acid (29.42%), L-malic acid (19.77%), quinic acid (15.51%), dibutyl phthalate (9.59%), and L-piperidic acid (7.18%) were relatively high. Nucleotides and their derivatives contained 47 substances, among which adenosine (37.9%), adenine (24.60%), 5-deoxy-5-methiadenosine (11.00%), 2’-deoxyinosine-5’-monophosphate (8.9%), and guanine (4.00%) were relatively high. Alkaloids include 4 pyridine alkaloids, 1 pyrrole alkaloid, 8 phenolamines, 3 piperidine alkaloids, 14 alkaloids and 13 indole alkaloids, including 1H-imidazo(4,5-D)pyridazine-2-amine (32.96%), fern lactam (12.11%), choline (11.34%), O-phosphocholine (6.89%), and histamine (6.77%). Terpenoids contained 5 kinds of terpenes and 36 kinds of triterpenoids, including 2-hydroxyursolic acid (13.87%), 3,24-dihydroxy-17,21-hemiacetal-12(13)oleanocarp (13.38%), 2,3,23-trihydroxyolean-12-en-28-acid methyl ester (8.33%), 3-hydroxylupin-20(29)-en-28-acid (betulinic acid) (6.5%), 3-hydroxyxyclopenolane-24-en-26-acid (isosperyl acid) (6.26%). There were 19 lignans and 20 coumarins in lignans and coumarins, among which the relative contents of epipinoresin* (18.15%), pineol* (17.43%), eugenylresinol-4’-O-glucoside (13.75%), podocarpus resinol (5.25%) and olivoresin-4,4’-di-O-glucoside (4.61%) were relatively high. Among the
quinoines, there were 7 anthraquinones, 1 phenanthrene and 5 quinones, among which chrysophanol-9-anthranoine (30.10%), 2,5-dimethoxybenzoquinone (21.52%), 3-hydroxy-1,2-dimethoxyanthraquinone (9.48%), 1,4-dimethoxy-2-hydroxyanthraquinone (8.69%), and 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone (7.24%) were relatively high. Tannin contained 2 kinds of tannin and 1 kind of proanthocyanadin, of which ellagic acid contained (51.17%), ellagic acid-4-O-glucoside (46%), and proanthocyanadinin B (2.83%). Among other classes, the relative contents of nicotinate ribonucleoside (64.98%), isonicotinic acid (11.75%), niacin (vitamin B3) (5.23%), orotate (vitamin B13) (4.55%), and menaquinone (vitamin K2) (3.74%) were relatively high.

4. Summary

The whole plant can be used as medicine, with a wide range of pharmacological effects, often used for anti-inflammatory, anti-tumor, antibacterial, enhance immune function and other effects. At present, there are few studies on the chemical composition of Serissa japonica whole grass, which mainly contains volatile oils, terpenes, lignans, and quinones [1]. There are few reports on other types of substances, and further exploration is needed. In this study, 12 types of substances were identified by extensive targeted metabolomics technology, among which the number of flavonoids, phenolic acids, amino acids and derivatives and lipid compound metabolites in the annotation results of primary metabolites was relatively large.

Flavonoids are polyphenolic compounds that are widely found in plants and have a variety of pharmacological effects. Flavonoids have antioxidant, anti-inflammatory, anti-tumor, hypolipidemic, and neuroprotective effects. It is often used for free radical scavenging, inhibiting inflammatory responses, inhibiting tumor cells, preventing and treating cardiovascular diseases, and treating neurological diseases [4]. A total of 77 flavonoids were identified in this study. Studies have shown that alfalfa is mainly used for anti-inflammatory, anti-tumor, antioxidant, and anti-aging effects. Flavonoids in grapes and strawberries can inhibit platelet aggregation, reduce LDL oxidation, and reduce oxidative stress and inflammation, thereby playing a cardiovascular role [5]. The baicalin and baicalin in Scutellaria baicalensis and chalcone in licorice [6] can play a bacteriostatic role, and the flavonoids in Rhiza sinensis have great value in the treatment of lung cancer and bowel cancer, and have become a hot spot in anti-tumor research [7]. In addition, there are many flavonoids with medicinal value, such as Shuxuening and Xindakang tablets, which contain flavonoids as the main ingredients [8].

Phenolic acids are compounds with a variety of biological activities and are widely found in plants. It has antioxidant, anti-inflammatory, antibacterial and anti-tumor effects. It is used to prevent and delay aging, prevent and treat bacterial infections, and resist tumor development. A total of 104 phenolic acids were identified in this study. Studies have shown that glucosyric acid can act as a small molecule inhibitor to affect PD1/PD-L1 protein interactions, thereby playing a role in the prevention and treatment of tumors.

Amino acids are the basic units that make up proteins and are also one of the essential nutrients for the human body. It can participate in a variety of metabolic pathways, biosynthesis and immune regulation, and plays an important role in the body's life activities. For example, L-arginine can maintain hepatocyte integrity and promote liver regeneration, and high doses of L-arginine can induce apoptosis of hepatocellular carcinoma cells, thereby inhibiting the growth of hepatocellular carcinoma. As an intermediate in drug synthesis, L-proline is widely used in the development of new drugs, such as L-proline, which is a promising and important raw material for the treatment of Parkinson's disease. L-pyroglutamic acid has anticonvulsant, anxiolytic and other effects.

Lipid compounds are involved in a variety of biological processes in living organisms, such as energy storage, cellular structural composition, and signal transduction as active components [10]. Studies have shown that lipid compounds play an important role in the treatment of metabolic syndromes, such as schizophrenia and rheumatoid arthritis, in addition to reducing the risk of cancer,
diabetes, and cardiovascular disease. Compared with traditional rosiglitazone in the treatment and prevention of diabetes, lipid compounds can improve the side effects of traditional drugs on myocardial injury, sphingolipids can inhibit the occurrence of colon cancer, lysphosphatidylethanolamine can be used as a drug carrier in anti-HIV and tumor , and can also increase the stability of the intestinal epithelial barrier for links or the treatment of enteritis or inflammatory bowel disease.

A large number of studies have shown that the whole herb has a variety of pharmacological effects, but which component dominates the role has not been explored. In addition, TCM does not rely on a single chemical substance to treat a certain disease, but rather relies on many chemical substances to coordinate with each other to exert pharmacological effects. In this study, the material components of *Serissa japonica* were studied by using a wide range of targeted metabolome technology, and the key substances that may play a role in *Serissa japonica* were discussed, which is of great significance for the basic research of pharmacodynamic substances and the rational development of resources, and to play a greater role in the national role.

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**References**


