Bioscience Evidence 2025, Vol.15, No.6, 280-290
http://bioscipublisher.com/index.php/be

Research Insight Open Access

Physiological and Ecological Mechanisms and Metabolic Pathway Analysis of Active Compound Accumulation in *Leonurus japonicus* Houtt.

Jiayao Zhou ✉, Hongpeng Wang
Traditional Chinese Medicine Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, China
✉ Corresponding email: jiayao.zhou@cuixi.org
Bioscience Evidence, 2025, Vol.15, No.6 doi: 10.5376/be.2025.15.0028
Received: 06 Oct., 2025
Accepted: 13 Nov., 2025
Published: 02 Dec., 2025

Preferred citation for this article:
Zhou J.Y., and Wang H.P., 2025, Physiological and ecological mechanisms and metabolic pathway analysis of active compound accumulation in *Leonurus japonicus* Houtt., Bioscience Evidence, 15(6): 280-290 (doi: 10.5376/be.2025.15.0028)

Abstract *Leonurus japonicus* Houtt. is a commonly used medicinal plant in traditional Chinese medicine. It contains many active ingredients, such as alkaloids, flavonoids and diterpenoids, so it is widely used in gynecology, cardiovascular and cerebrovascular diseases, and anti-inflammation, etc. In recent years, multi-omics studies and molecular biology research have gradually clarified how these major active components (such as leonurine, tribulus terephthine, diterpenoids, etc.) accumulate in different organs and under different environments. At the physiological level, some key enzymes (such as ADC, UGT, and SCPL) are very important. The gene clusters they belong to have been amplified and new functions have emerged. These changes have made the synthesis of active ingredients smoother and also given them more obvious accumulation characteristics in plants. Environmental factors can also have an impact, such as pH, climate, soil and geographical location. These conditions can change the supply of substrates and the activity of enzymes, thereby affecting the content of active ingredients. The study of metabolic pathways can not only assist in molecular breeding and more precise cultivation, but also provide a scientific basis for the quality control of medicinal materials and the development of new drugs. In the future, under the perspective of systems biology, integrating multi-omics data and combining it with gene function verification and ecological adaptation research is expected to promote more efficient molecular regulation of the active components of Leonurus japonicus. At the same time, it can also facilitate the sustainable utilization of resources and the modern development of their medicinal value.
Keywords *Leonurus japonicus*; Accumulation of active ingredients; Physiological mechanism; Ecological regulation; Metabolic pathways

1 Introduction

Leonurus japonicus Houtt. Is an annual or biennial herb of the Lamiaceae family and is very common in China, South Korea, Japan, Southeast Asia and other places. It has been used in traditional Chinese medicine for a long time, especially in gynecology, and is listed as a superior grade in the "Shennong's Classic of Materia Medica". It is commonly used in clinical practice to regulate menstruation, promote blood circulation, diuresis and reduce swelling, and is also used for some cardiovascular problems (He et al., 2024). Modern research has found that *Leonurus japonicus* contains many active ingredients, such as leonurine, tribulus terrestris and other alkaloids, as well as flavonoids and diterpenoids. These components have functions such as regulating the uterus, protecting the cardiovascular system, anti-inflammation, anti-oxidation and anti-tumor. More than 140 compounds have been identified in its aboveground parts and fruits so far, mainly alkaloids, flavonoids and terpenoids.

The efficacy of motherwort mainly depends on the types and contents of these active components, and these components are affected by multiple aspects such as genetics, environment, organ differentiation and physiological state (Tan et al., 2020; Han et al., 2023; Chen et al., 2024). Under different origins, different organs and different growth conditions, the contents and compositions of these components may vary greatly, thereby affecting the quality of medicinal materials and clinical effects. In addition, some ecological factors, such as climate and soil pH, can also regulate the synthesis and accumulation of secondary metabolites (Zhang et al., 2022). Therefore, a deeper understanding of the accumulation mechanism of active ingredients in *Leonurus japonicus* can help us select a more suitable cultivation environment, improve the quality of medicinal materials, and also provide a theoretical basis for new drug development and sustainable resource utilization.

This review mainly aims to systematically sort out how these active components in *Leonurus japonicus* (such as leonurine, tribulus terline, flavonoids, diterpenoids, etc.) accumulate under different physiological and ecological conditions. The key contents include their metabolic pathways, key enzymes and genes, environmental regulatory factors, and related molecular regulatory networks. This review covers multi-omics studies such as genomics, transcriptomics and metabolomics, the distribution of components in different organs and origins, as well as the influence of environmental factors such as climate and soil pH on component synthesis. At the same time, the latest research progress of related metabolic pathways was also introduced, hoping to provide a theoretical basis and new ideas for improving the quality of *Leonurus japonicus* medicinal materials, molecular breeding and new drug development.

2 Overview of Active Ingredients in *Leonurus japonicus*

2.1 Chemical category and biosynthetic sources

More than 140 components have been isolated from *Leonurus japonicus*. Including alkaloids (such as leonurine, stachydrine, trigonelline), diterpenoids (mainly labdane type), flavonoids, lignans, phenolic acids, polysaccharides, etc. (Zhao et al., 2022). Among them, alkaloids and diterpenoids are the most typical and extensively studied type of components (Miao et al., 2019).

Among alkaloids, leonurine is the most representative one, and it has a rather unique synthetic route. The latest multi-omics research shows that this route requires enzymes such as arginine decarboxylase (ADC), UDP-glucosyltransferase (UGT), and SCPL acyltransferase to be completed, and the evolution of these gene clusters enables leonurine to accumulate more specifically in plants (Li et al., 2023) (Figure 1). Tribulus terrestris and trihydroxy alkaloids are equally common and can be detected in different parts of plants (Zhang et al., 2021; 2025).

Among diterpenoids, Labdane-type structures are relatively common, such as furanolabdane, lactonelabdane, seco-labdane, etc. (Zhang et al., 2020; Cao et al., 2023; Wei et al., 2023). These diterpenoids are mainly synthesized through the MEP pathway and often accumulate in the aboveground parts of plants, especially in glandular hairs (Xiao et al., 2017). Flavonoids (such as quercetin, kaempferol) and lignans (such as some sesquineolignan glycosides and tetrahydrofuran lignans) can also be found in *Leonurus japonicus*, and they usually have antioxidant, anti-inflammatory and other effects (Tian et al., 2021; Bu et al., 2024). Motherwort polysaccharides are mainly composed of glucose, fucose, mannose, etc., and exhibit antioxidant and melanin-inhibiting activities (Zhang et al., 2023). Phenolic acids such as caffeic acid and chlorogenic acid also have certain pharmacological effects (Tan et al., 2020).

2.2 Biological activity and pharmacological correlation

These active components of *Leonurus japonicus* can play multiple roles in the body and also form the basis for it to be used as a medicinal material. In terms of alkaloids, Leonuronine can regulate menstruation, promote blood circulation, and has effects such as cardiovascular and cerebrovascular protection, antioxidation, anti-inflammation and neuroprotection. Tribulus terrestris also has cardiovascular protective and anti-tumor effects and can affect uterine function. Trihydroxy bases can inhibit mast cell activation and relieve asthma and inflammation (Zhang et al., 2021). Diterpenoid components usually have functions such as anti-inflammation, anti-tumor, anti-thrombosis and neuroprotection. Some of these components exert their effects by inhibiting signaling pathways such as NF-κB and MAPK (Wei et al., 2023). Flavonoids and lignans also have antioxidant, anti-inflammatory and liver-protective effects. *Leonurus japonicus* polysaccharides exhibit antioxidant, melanin-inhibiting and cytoprotective effects.

2.3 Tissue and developmental distribution

The distribution of the active ingredients of motherwort varies in different tissues and at different developmental stages. In terms of tissue distribution, diterpenoids mainly accumulate in the aboveground parts, especially in glandular hair structures (Xiao et al., 2017). Alkaloids such as leonurine and tribulus terrestris are present in stems, leaves and flowers, but the content varies depending on the part. Lignans are more frequently found in fruits (Tian et al., 2021). During the development process, the content and combination of components will also change along with the growth of the plant, which will affect the quality of the final medicinal materials (Zhao et al., 2022). In addition, different origins and ecological conditions can also affect the accumulation mode of these components.

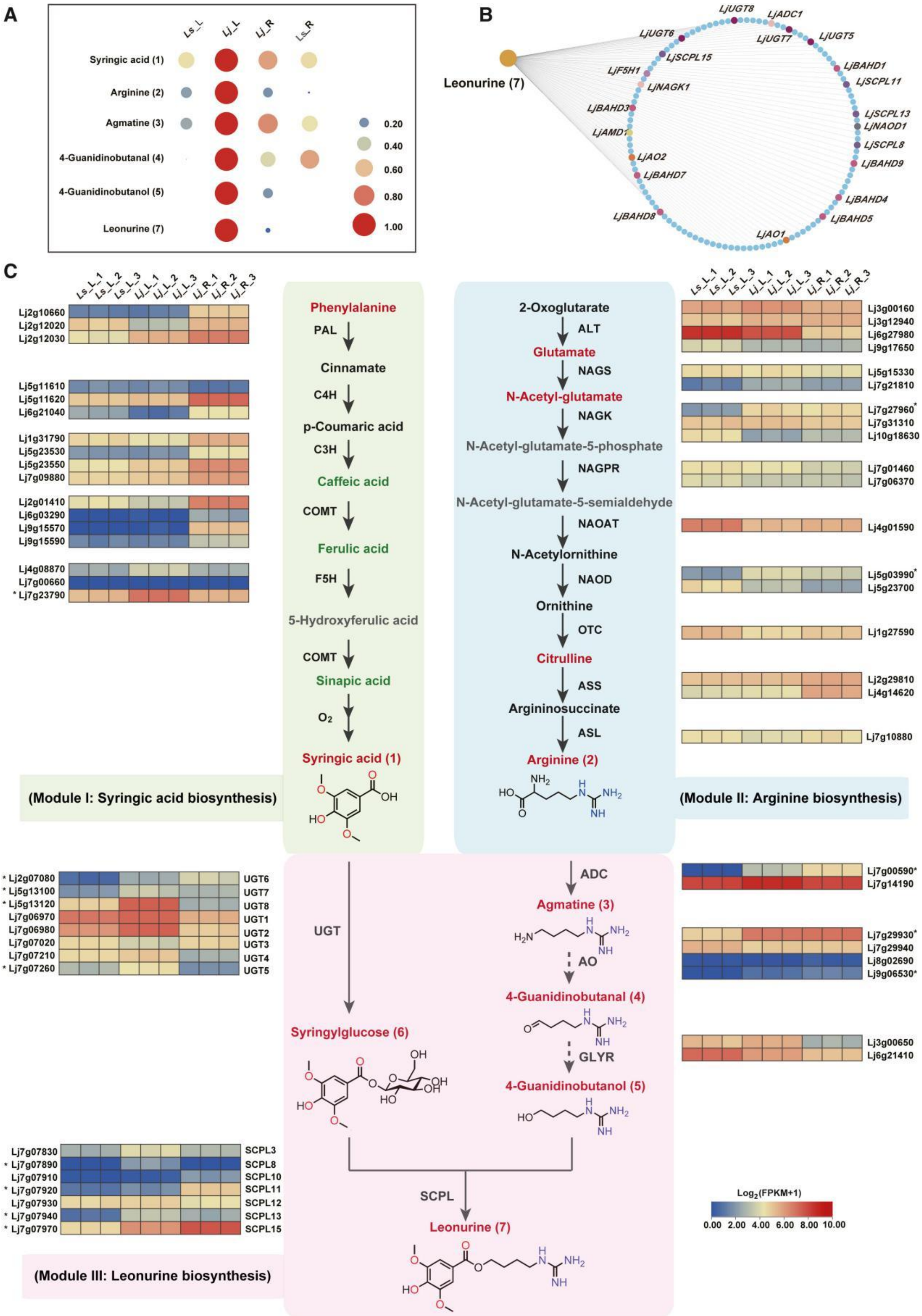

Figure 1 Construction of the leonurine biosynthesis pathway through integrated multiomics analyses (Adopted from Li et al., 2023)

3 The Physiological Regulatory Mechanism of Active Ingredient Accumulation in *Leonurus japonicus*

3.1 Photosynthesis, carbon and nitrogen allocation and precursor supply

The synthesis and accumulation of the active components of motherwort cannot do without the substances and energy provided by photosynthesis, nor can it do without the balance of carbon and nitrogen metabolism. Photosynthesis provides a carbon framework and energy for secondary metabolites such as alkaloids and diterpenoids, and nitrogen is an important source of nitrogen-containing components like alkaloids. Studies have found that under different pH conditions, if the contents of photosynthetic pigments and soluble proteins in leaves increase, the growth and yield of plants will improve, and there will also be more "raw materials" for the synthesis of active components. Especially the synthesis of alkaloids, such as tribulus terrestris, which is closely related to the assimilation and transport of nitrogen. Under high pH conditions, the substrates and products related to the nitrogen addition reaction increase, thereby promoting the formation of alkaloids.

3.2 Hormone regulation

Plant hormones have a significant impact on the accumulation of active ingredients in motherwort. Guo et al. (2025) discovered in transcriptome research that under drought conditions, the expression of genes related to hormone signals undergoes significant changes, especially the ABA (abscisic acid) pathway is significantly activated. Among them, ABA can affect the WRKY transcription factor and MAPK signaling pathway, indirectly regulating the synthesis and accumulation of active components in *Leonurus japonicus*. During this period, hormone signals will also interact with environmental stress to increase the activity of the biosynthetic pathway of active ingredients and help plants cope with adverse conditions more smoothly.

3.3 Enzyme activity and rate-limiting steps

In the process of synthesizing active ingredients from *Leonurus japonicus*, various enzymes are required, some of which are rate-limiting steps and have a significant impact on the accumulation of components in *Leonurus japonicus*. Li et al. (2023) conducted a series of studies taking leonurine as the research object. They found that the synthesis process of leonurine mainly requires several enzymes, namely ADC, UGT and SCPL. Through genomic and multi-omics studies, they also found that the massive accumulation of leonurine was mainly attributed to the amplification of the UGT-SCPL gene cluster and the emergence of new functions.

3.4 Stress physiology and induction

Environmental changes often cause motherwort to produce more active ingredients. In a dry environment, the contents of malondialdehyde, proline and hydrogen peroxide in *Leonurus japonicus* plants will all increase. The expression levels of transcription factors such as WRKY will also increase. These changes will enhance the antioxidant response of plants and increase the synthesis of some secondary metabolites. Guo et al. (2025) KEGG analysis also indicated that under drought conditions, the MAPK signaling pathway, hormone signaling pathways, and some genes related to secondary metabolism would significantly increase, thereby promoting the generation of diterpenoids and other components. Zhang et al. (2022) pointed out that changes in pH can affect nitrogen metabolism and the levels of some substrates, and also increase alkaloids such as tribulus terrestris. These physiological responses together enhance the stress tolerance of *Leonurus japonicus* and also increase the content of its medicinal components.

4 Ecological and Environmental Driving Factors of the Metabolic Profile of Active Ingredients in *Leonurus japonicus*

4.1 Soil characteristics

The texture of the soil, the content of organic matter, water retention capacity and other multiple factors jointly affect the growth of motherwort and the accumulation of nutrients. Chen et al. (2024) concluded that the Available Water Capacity (AWC) of the soil, the portion of water that plants can actually utilize in the soil, is a key factor influencing the distribution and adaptability of motherwort. The efficiency of plants in absorbing water and nutrients varies with AWC, and the synthesis of its active components also changes accordingly. Other properties of the soil, such as pH value, fertility level, and the structure of microbial communities, can also indirectly change the types and contents of secondary metabolites by influencing root metabolism and signal regulation.

4.2 Light, temperature and altitude

Environmental factors such as light intensity, light duration, air temperature and altitude profoundly affect the growth range and active ingredient production of motherwort. The core climatic factors for choosing an environment suitable for the growth of motherwort are the highest temperature, the lowest temperature and the altitude of the area where it is located. These factors play a crucial role in the growth of motherwort. Bai et al. (2025) and Hu et al. (2025) conducted mitochondrial genome research. They found that genes such as NADH dehydrogenase and ATP synthase, which are directly related to energy metabolism, would be subject to relatively obvious purification selection under different light and temperature conditions. This indicates that motherwort has a relatively strong ability to adapt to environmental changes. In areas with high altitude and large temperature differences between day and night, plants tend to adjust respiration and antioxidant responses, thereby increasing the content of certain active components.

4.3 Moisture conditions and seasonal variations

Precipitation and soil moisture supply also have a significant impact on the growth of motherwort. The precipitation in the warm season and the precipitation in the driest month will directly affect the ecological suitability of motherwort (Wang et al., 2023). Drought inhibits plant growth, causing plants to activate some genes that regulate stress responses, including WRKY transcription factors and MAPK signaling pathways, thereby increasing the production of secondary metabolites such as alkaloids and diterpenoids. The changes in precipitation and temperature during the seasons will also cause the content of active ingredients to fluctuate up and down with the seasons, thereby affecting the quality and efficacy of motherwort medicinal materials.

4.4 Geographical origin and ecological differentiation

There are also obvious differences among motherwort from different origins and with different ecological types. Han et al. (2023) and Zhang et al. (2025) have conducted research on this phenomenon. Their research conclusions all indicate that there are significant differences in genotype and active ingredient content among motherwort from different regions. Some of these differences stem from genetic background, while others are influenced by environmental factors. Zhang et al. (2025) analyzed motherwort from Zhejiang in their research and found that the content of tribulus terrestris in motherwort from Zhejiang was higher than that in other places. Historical climate events such as distribution contraction and expansion during the ice age have also shaped the current distribution pattern and genetic diversity of motherwort.

5 The Metabolic Pathways of the Main Active Components of *Leonurus japonicus*

5.1 Alkaloid biosynthesis: Leonurine and *Tribulus terrestris*

Leonurine and stachydrine are the two most typical alkaloids in *Leonurus japonicus*. According to the multi-omics study by Li et al. (2023), the synthesis of leonurine uses arginine as the starting substrate and requires the participation of key enzymes such as arginine decarboxylase (ADC), UDP-glucosyltransferase (UGT), and SCPL acyltransferase. Among them, the amplification of the UGT-SCPL gene cluster and the generation of new functions are important reasons why leonurine can accumulate in large quantities in plants. The synthesis of tribulus terrestris base is related to nitrogen addition reactions, including rate-limiting steps such as reductive amination and Schiff base formation. Zhang et al. (2022) and He et al. (2024) both conducted research and found that under alkaline conditions, the substrates and products required for these reactions are more likely to accumulate, thereby enhancing the synthesis efficiency of terrestris alkaloids. Although the entire metabolic pathway of tribulus terrestris is not yet fully understood, the general framework is relatively clear.

5.2 Phenylpropane/flavonoids metabolic pathway

Phenylpropanes and flavonoids are important secondary metabolites in *Leonurus japonicus*, both of which have antioxidant and anti-inflammatory effects. The phenylpropane pathway starts with phenylalanine and, through the catalysis of enzymes such as PAL, C4H and 4CL, can further form various phenolic and flavonoid substances. MYB transcription factors are the main regulators of this metabolic pathway. They can regulate the expression of structural genes, thereby influencing the synthesis of flavonoids, anthocyanins and lignin, and are also involved in plant growth and stress response (Pratyusha and Sarada, 2022). Some flavonoids in *Leonurus japonicus*, such as apigenin and apigenin-7-methyl ether, can exert pharmacological effects by inhibiting key enzymes such as aromatase (Shi et al., 2024).

5.3 Metabolic pathways of terpenoids and diterpenoids

Motherwort contains many terpenoids and diterpenoids, among which Spiro-9, 13-epoxy-Labdane-type diterpenoids are the most representative. Transcriptome and functional studies have found that *Leonurus japonicus* contains six diterpene synthases (diTPSs), including three types II (LjTPS1, LjTPS3, LjTPS4) and three types I (LjTPS5, LjTPS6, LjTPS7). Co-catalyzed the formation of the SPIRo-9, 13-epoxy labdane skeleton (Wang et al., 2022). Among them, LjTPS3 is responsible for generating C9-hydroxylated bicyclic PPP, while LjTPS6 can further generate various LabDane-like diterpenoids. Structural and functional studies have indicated that if mutations occur at the I420 site of LjTPS6, it will affect the generation of 9,13 s-epoxy-labDANE, providing a molecular basis for modifying the diterpene metabolic pathway. Genomic analysis also indicated that the gene family related to diterpene biosynthesis in *Leonurus japonicus* was significantly amplified.

5.4 Multi-omics integration analysis

Multi-omics integration (including genomic, transcriptomic, metabolomic and enzyme activity analysis) is an important method for studying the metabolic network of motherwort. Through high-quality genome assembly and combined with transcriptome and metabolome data, researchers reconstructed the complete metabolic pathways of major components such as leonurine, and also identified the evolutionary and functional differences of key genes. Multi-omics data can help identify new metabolic branches, regulatory genes and rate-limiting enzymes, providing a theoretical basis for molecular breeding and synthetic biology research of medicinal components (Jamil et al., 2020; Wang et al., 2024) (Figure 2). Molecular breeding and synthetic biology provide a theoretical basis.

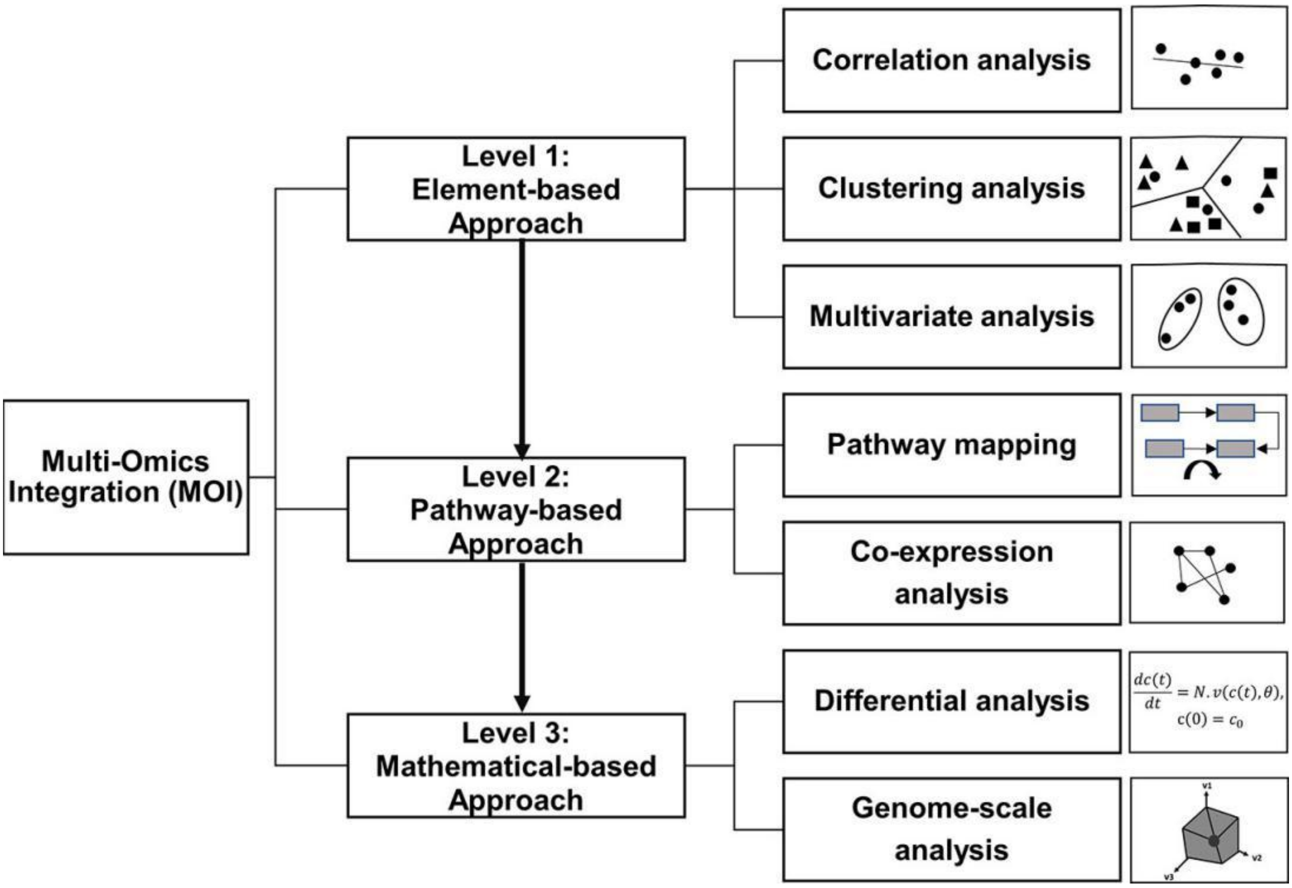

Figure 2 Current approaches in multi-omics integration (MOI) of plant systems biology. This MOI strategy is classified into three main levels with increasing degrees of complexity (Adopted from Jamil et al., 2020)

6 An Integrated Analysis Case of Leonurine accumulation in Different Ecological Types of *Leonurus japonicus*

6.1 Background and sampling strategy

leonurine is a characteristic component of *Leonurus japonicus*, and its content varies greatly among different ecological types. To better study these differences, relevant research usually selects ecological types with high leonurine content (such as *L. japonicus*) and low leonurine content (such as *L. sibiricus*) as representatives, and then collects samples from different regions. When sampling, plants growing in different environments will be selected, and then a comprehensive analysis will be conducted by combining the genome, transcriptome and metabolome (Li et al., 2023).

6.2 Physiological indicators

In different ecological types or under stress conditions such as drought, motherwort will undergo some physiological changes. For instance, indicators such as malondialdehyde (MDA), proline and hydrogen peroxide (H_2O_2) can change, and these indicators are often used to reflect the stress resistance of plants. And these abilities are closely related to the accumulation of secondary metabolites such as leonurine.

6.3 Ecological factors

There are many ecological factors that affect the content of leonurine, including temperature, precipitation, soil moisture, geographical location and local ecological suitability. Research shows that indicators such as warm season precipitation, average temperature in colder seasons, and soil available water capacity all affect the distribution of *Leonurus japonicus* and the accumulation of leonurine. Generally speaking, areas with high ecological suitability are more likely to accumulate more leonurine (Chen et al., 2024).

6.4 Metabolomics and transcriptomic results

The synthesis of leonurine requires the joint participation of key enzymes such as ADC, UGT and SCPL. In ecological plants with high leonurine content, the UGT-SCPL gene cluster will show amplification and is more likely to generate new functions, making it easier for leonurine to accumulate. In addition, Li et al. (2023) and Guo et al. (2025) reached similar conclusions after conducting transcriptome analysis on *Leonurus japonicus* in their research: under stress such as drought, transcription factors like WRKY in *Leonurus japonicus* plants increase significantly, regulating the gene expression of secondary metabolic pathways and promoting the generation of active components such as leonurine.

6.5 Mechanism integration interpretation

The differences in leonurine content among different ecological types are the result of the combined effect of multiple factors, including genomic structural differences (such as the amplification of UGT-SCPL gene clusters), the strength of transcriptional regulation, and the way plants adapt to the environment, etc. In areas with better ecological suitability, plants tend to better regulate physiological indicators related to stress resistance, activate secondary metabolic pathways, and produce more leonurine.

6.6 Implications for cultivation and breeding

This case provides a reference for the screening of high-quality germplasm of motherwort, the targeted cultivation of different ecological types, and molecular breeding. If the cultivation is carried out in areas with high ecological suitability and the germplasm with UGT-SCPL amplification genotypes is given priority, the content of leonurine can be significantly increased, making the quality of the medicinal materials more stable. Meanwhile, multi-omics technology can also help rapidly screen superior varieties and promote molecular marker-assisted breeding (Li et al., 2023).

7 Application Prospects: Moving Towards the Quality Improvement and Metabolic Engineering of Active Ingredients in Motherwort

7.1 Ecological cultivation strategies

Ecological suitability is an important foundation for enhancing the content of active components and the quality of *Leonurus japonicus* as a medicinal material. Climatic conditions, such as the precipitation in the warm season and the average temperature in the cold season, as well as the effective water capacity of the soil and other factors, will significantly affect the distribution of motherwort and the amount of its medicinal components. By conducting ecological suitability zoning for these environmental factors or using climate change models, more suitable areas can be selected for targeted planting, thereby increasing the content of components such as leonurine. The active ingredients of motherwort from different origins also vary significantly. For instance, the contents of leonurine and tribulus terrestris in the plants from Zhejiang region are relatively high. This indicates that choosing the appropriate origin and doing a good job in ecological management are very crucial for the quality of medicinal materials (Zhang et al., 2025).

7.2 Molecular breeding and gene editing

High-quality genomic and multi-omics data provide a reliable basis for the molecular breeding and gene editing of motherwort. Studies have found that the synthesis of leonurine is inseparable from key enzymes such as ADC, UGT and SCPL, and the amplification and functional changes of gene clusters related to these enzymes are important genetic reasons for the large accumulation of its active ingredients (Wang et al., 2024). Germplasm resources with highly active component genotypes can be screened out through molecular marker-assisted selection (MAS) or genome-wide association analysis (GWAS). Gene editing technologies, such as CRISPR/Cas9, are also expected to directly regulate the genes of these key enzymes, further enhancing the synthesis efficiency of components such as leonurine (Bai et al., 2025). The research on mitochondrial genomes and repeat sequences also provides a new reference direction for genetic diversity conservation and breeding (Hu et al., 2025).

7.3 Synthetic biology and heterologous production

Synthetic biology provides a new approach for the heterologous production of active ingredients from *Leonurus japonicus*. By analyzing the complete metabolic pathways of components such as leonurine and reconstructing these pathways in model organisms such as yeast or Arabidopsis thaliana, it is expected to achieve large-scale and controllable production of these medicinal components (Li et al., 2023). The identification of high-quality genomes and functional genes provides key genes and regulatory elements for the construction of synthetic biology platforms (Wang et al., 2024). In addition, heterologous expression systems can not only be used to verify gene functions but also for high-throughput screening, laying the foundation for new drug development and industrialization.

8 Challenges and Future Directions

8.1 Deficiencies in gene function identification

Although there have been many advancements in the genomic, transcriptomic and multi-omics studies of *Leonurus japonicus* in recent years, the functions of many key genes have actually not been clarified. For instance, we already know that enzymes such as ADC, UGT, and SCPL are very important in the synthesis of leonurine, but their regulatory methods under different environments, changes in enzyme activity, and the mutual influences among them are still not clear enough. In addition, although there are some clues about the role of transcription factors like WRKY in stress response and secondary metabolism regulation, their downstream target genes and regulatory networks still need to be further verified (Li et al., 2023). Mitochondrial and chloroplast genome studies have also provided considerable information for breeding and phylogeny, but their specific contributions to the accumulation of active components still need to be further explored.

8.2 Understanding of ecological interaction mechanisms

The amount of active ingredients in *Leonurus japonicus* is not only related to genes, but also closely related to the ecological environment. Climate, soil, moisture and geographical location all affect its distribution and the content of medicinal components (Wang et al., 2023). But at present, we still don't know much about the specific relationship between it and these environmental factors, such as how changes in water, temperature and pH affect the plants? What effects do the microorganisms in the rhizosphere and the surrounding companion plants have? All of these require more systematic research. Although ecological suitability zoning and climate simulation have provided a basis for resource conservation and cultivation, research on the ecological interaction mechanism itself is still insufficient.

8.3 Standardization of cultivation and quality control

Under different production areas and different planting conditions, the active components of *Leonurus japonicus* often vary greatly, which can affect the quality of the medicinal materials and also the clinical effects (Zhang et al., 2025). Most of the current quality control methods only measure one or a few components, such as leonurine or tribulus terrestris, lacking a systematic assessment approach that encompasses multiple components and indicators. Under different parts and storage methods, whether the active ingredients are stable and how they are related to the efficacy still need further study. Furthermore, standardized cultivation, harvesting, processing and quality evaluation systems have not been fully established yet, all of which have restricted the further development of the industry.

In the future, it is necessary to enhance the systematic identification and functional verification of key genes, and combine multi-omics and gene editing technologies to more deeply analyze the regulatory networks of active ingredients. At the same time, further research should be conducted on the relationship between L. japonicus and environmental factors to clarify the ecological interaction mechanism, providing support for targeted cultivation and resource conservation. It is also necessary to establish more comprehensive quality control standards, including multi-index and multi-level assessment systems, and promote the standardization of cultivation, harvesting and processing, laying a foundation for the high-quality utilization and modern application of *Leonurus japonicus* as a medicinal material.

9 Conclusion

The active ingredients of *Leonurus japonicus* are jointly affected by various physiological and ecological factors. In terms of physiology, key enzymes such as ADC, UGT, and SCPL, as well as their related gene clusters, if they undergo amplification or develop new functions, will directly promote the synthesis and accumulation of major components such as leonurine and tribulus terrestris. Meanwhile, under stress conditions such as drought, transcription factors like WRKY will be involved in regulating secondary metabolic pathways, thereby influencing the generation of these active substances. In terms of ecological environment, conditions such as precipitation, temperature, soil pH and moisture not only determine whether motherwort can grow in a certain area, but also affect the content and distribution pattern of active components. The differences in origin and environment can bring about genetic and metabolic changes, which in turn affect the quality of medicinal materials.

The research on the metabolic pathways of the active components of *Leonurus japonicus* provides a foundation for molecular breeding, precise cultivation and medicinal development. High-quality genomic and multi-omics data enable researchers to identify key metabolic genes more quickly and verify their functions, and also provide clear targets for molecular marker-assisted breeding and gene editing. Clear metabolic pathways can also help us efficiently produce these components in heterologous systems such as yeast, alleviating the problem of insufficient natural sources. Meanwhile, these research achievements also provide reliable basis for the quality control of medicinal materials and the development of new drugs.

Future research should continue to take systems biology as the main line, integrating multi-level data such as the genome, transcriptome, metabolome and phenome to more comprehensively analyze the regulatory network of active components. Combining ecological adaptability research with environmental simulation can promote more precise ecological cultivation and resource protection. Under the framework of systems biology, molecular breeding, synthetic biology and medicinal development can also develop in a better coordinated manner, helping to achieve high-quality and sustainable utilization of *Leonurus japonicus* as a medicinal material, and providing new directions for the modernization of traditional Chinese medicine and the research and development of new drugs.

Acknowledgments

We sincerely thank our supervisor, Professor Cai, for your meticulous guidance and selfless assistance throughout the research process

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Bai X., Zhu T., Chen H., Wang X., Liu J., Feng Y., Huang Y., Lee J., Kokubugata G., Qi Z., and Yan X., 2025, Unraveling the mitochondrial genome of the medicinal Chinese motherwort (*Leonurus japonicus*, Lamiaceae): structural dynamics, organelle-to-nuclear gene transfer, and evolutionary implications, Frontiers in Plant Science, 16: 1546449.
https://doi.org/10.3389/fpls.2025.1546449

Bu L., Zhou Q., Peng C., Shu H., Zhou F., Wu G., Liu F., Tian H., and Xiong L., 2024, New sesquineolignan glycoside isomers from the aerial parts of *Leonurus japonicus* and their absolute configurations, Arabian Journal of Chemistry, 17(9): 105932.
https://doi.org/10.1016/j.arabjc.2024.105932

Cao X., Wang X., Zhang Y., Xu D., Song X., Yu J., Bao J., Zhang J., and Zhang H., 2023, New di-spirocyclic labdane diterpenoids from the aerial parts of *Leonurus japonicus*, Chinese Journal of Natural Medicines, 21(7): 551-560.
https://doi.org/10.1016/s1875-5364(23)60446-7

Chen C., Zhou L., Han Y., Wen J., Liu L., Liu Q., Peng C., and He Y., 2024, Comprehensive analysis of the effects of climate change on the species distribution and active components of *Leonurus japonicus* Houtt, Industrial Crops and Products, 218: 119017.
https://doi.org/10.1016/j.indcrop.2024.119017

Guo T., Wang Q., Yi Y., Yin Z., Xu L., Yang F., Xu J., and Zhang M., 2025, Transcriptome analysis and genome-wide identification of WRKY gene family in *Leonurus japonicus* under drought stress, BMC Plant Biology, 25(1): 607.
https://doi.org/10.1186/s12870-025-06606-7

Han Y., Wen J., Chen C., Zhou L., Zhang T., Feng W., Zhao Z., and He Y., 2023, Relevance of genetic and active ingredient content differences in *Leonurus japonicus* Houtt from different origins, Genetic Resources and Crop Evolution, 71(2): 801-810.
https://doi.org/10.1007/s10722-023-01660-2

He Z., Li P., Liu P., and Xu P., 2024, Exploring stachydrine: from natural occurrence to biological activities and metabolic pathways, Frontiers in Plant Science, 15: 1442879.
https://doi.org/10.3389/fpls.2024.1442879

Hu Q., Li Q., Mao Y., Luo Y., Deng Z., and Zhang W. (2025). Assembly and analysis of the complete mitochondrial genome of *Leonurus japonicus* (Lamiaceae). Scientific Reports, 15.
https://doi.org/10.1038/s41598-025-97594-z

Jamil I., Remali J., Azizan K., Muhammad N., Arita M., Goh H., and Aizat W., 2020, Systematic Multi-Omics Integration (MOI) Approach in Plant Systems Biology, Frontiers in Plant Science, 11: 944.
https://doi.org/10.3389/fpls.2020.00944

Li P., Yan M., Liu P., Yang D., He Z., Gao Y., Jiang Y., Kong Y., Zhong X., Wu S., Yang J., Wang H., Huang Y., Wang L., Chen X., Hu Y., Zhao Q., and Xu P., 2023, Multi-Omics Analyses of Two Leonurus Species Illuminate Leonurine Biosynthesis and Its Evolution, Molecular Plant, 17(1): 158-177.
https://doi.org/10.1016/j.molp.2023.11.003

Miao L., Zhou Q., Peng C., Liu Z., and Xiong L., 2019, *Leonurus japonicus* (Chinese motherwort), an excellent traditional medicine for obstetrical and gynecological diseases: A comprehensive overview, Biomedicine and Pharmacotherapy = Biomedecine and Pharmacotherapie, 117: 109060.
https://doi.org/10.1016/j.biopha.2019.109060

Pratyusha D., and Sarada D., 2022, MYB transcription factors-master regulators of phenylpropanoid biosynthesis and diverse developmental and stress responses, Plant Cell Reports, 41: 2245-2260.
https://doi.org/10.1007/s00299-022-02927-1

Shang X., Pan H., Wang X., He H., and Li M., 2014, *Leonurus japonicus* Houtt.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine, Journal of Ethnopharmacology, 152(1): 14-32.
https://doi.org/10.1016/j.jep.2013.12.052

Shi X., Peng T., Azimova B., Li X., Li S., Cao D., Fu N., Zhang G., Xiao W., and Wang F., 2024, Luteolin and its analog luteolin-7-methylether from *Leonurus japonicus* Houtt suppress aromatase-mediated estrogen biosynthesis to alleviate polycystic ovary syndrome by the inhibition of tumor progression locus 2, Journal of Ethnopharmacology, 331: 118279.
https://doi.org/10.1016/j.jep.2024.118279

Tan Y., Xu D., Yue S., Tang Y., Guo S., Yan H., Zhang J., Zhu Z., Shi X., Chen Y., Gu Y., Ding X., Huang S., Peng G., Zhou G., and Duan J., 2020, Comparative analysis of the main active constituents from different parts of *Leonurus japonicus* Houtt. and from different regions in China by ultra-high performance liquid chromatography with triple quadrupole tandem mass spectrometry, Journal of Pharmaceutical and Biomedical Analysis, 177: 112873.
https://doi.org/10.1016/j.jpba.2019.112873

Tian Z., Liu F., Peng F., He Y., Shu H., Lin S., Chen J., Peng C., and Xiong L., 2021, New lignans from the fruits of *Leonurus japonicus* and their hepatoprotective activities, Bioorganic Chemistry, 115: 105252.
https://doi.org/10.1016/j.bioorg.2021.105252

Wang J., Mao Y., , Y., Yang J., Jin B., Lin H., Tang J., Zeng W., Zhao Y., Gao W., Peters R., Guo J., Cui G., and Huang L., 2022, Diterpene synthases from *Leonurus japonicus* elucidate epoxy-bridge formation of spiro-labdane diterpenoids, Plant Physiology, 189(1): 99-111.
https://doi.org/10.1093/plphys/kiac056

Wang X., Zhang L., Yao G., Wang X., Yi S., Meng T., Meng D., Chen W., and Guo L., 2024, De novo chromosome-level genome assembly of Chinese motherwort (*Leonurus japonicus*), Scientific Data, 11(1): 55.
https://doi.org/10.1038/s41597-023-02901-w

Wang Y., Xie L., Zhou X., Chen R., Zhao G., and Zhang F., 2023, Prediction of the potentially suitable areas of *Leonurus japonicus* in China based on future climate change using the optimized MaxEnt model, Ecology and Evolution, 13(10): e10597.
https://doi.org/10.1002/ece3.10597

Wei Q., Cao X., Xu D., Wang S., Zhang J., and Zhang H., 2023, Anti-inflammatory labdane diterpenoids from the aerial parts of *Leonurus japonicus*, Phytochemistry, 210: 113646.
https://doi.org/10.1016/j.phytochem.2023.113646

Xiao C., Liu Y., Luo S., Hua J., Liu Y., and Li S., 2017, Localisation of Two Bioactive Labdane Diterpenoids in the Peltate Glandular Trichomes of *Leonurus japonicus* by Laser Microdissection Coupled with UPLC-MS/MS, Phytochemical analysis : PCA, 28(5): 404-409.
https://doi.org/10.1002/pca.2687

Zhang K., Meng C., Liu J., Zhang L., Peng C., and Xiong L., 2025, Recognition and Comprehensive Quantitation of Stachydrine Analogues in *Leonurus japonicus* Based on HILIC-QTOF MS and qHNMR, Analytical Chemistry, 97(1): 535-542.
https://doi.org/10.1021/acs.analchem.4c04751

Zhang L., Khoo C., Xiahou Z., Reddy N., Li Y., Lv J., Sun M., Fan H., and Zhang X., 2023, Antioxidant and anti-melanogenesis activities of extracts from *Leonurus japonicus* Houtt, Biotechnology and Genetic Engineering Reviews, 40: 2888-2909.
https://doi.org/10.1080/02648725.2023.2202544

Zhang W., Zhang Y., Chen S., Zhang H., Yuan M., Xiao L., Lu Y., and Xu H., 2021, Trigonelline, An Alkaloid From *Leonurus japonicus* Houtt., Suppresses Mast Cell Activation and OVA-Induced Allergic Asthma, Frontiers in Pharmacology, 12: 687970.
https://doi.org/10.3389/fphar.2021.687970

Zhang X., Zhong W., Liu R., Wang Y., Luo T., Zou Y., Qin H., Li X., Zhang R., and Xiao W., 2020, Structurally Diverse Labdane Diterpenoids from *Leonurus japonicus* and Their Anti-inflammatory Properties in LPS-Induced RAW264.7 Cells, Journal of Natural Products, 83(9): 2545-2558.
https://doi.org/10.1021/acs.jnatprod.9b00597

Zhang Y., Cui X., Wang W., Hou J., and Yan B., 2022, Effects of pH value on stachydrine biosynthesis of hydroponic *Leonurus japonicus* and its physiological mechanism, Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica, 47(20): 5502-5507.
https://doi.org/10.19540/j.cnki.cjcmm.20220712.101

Zhao Z., Yao Z., Lin S., Chu G., Mu K., Wang Y., Bi K., Wang T., Li Q., and Liu R., 2022, *Leonurus japonicus* Houtt. (Motherwort): Systematic research through chemical profiling, stability under controlled conditions and pharmacokinetic analysis on screening Q-markers for quality control, Journal of Pharmaceutical and Biomedical Analysis, 213: 114707.
https://doi.org/10.1016/j.jpba.2022.114707