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Variation in Secondary Metabolite Traits of *Leonurus japonicus* and Their Functional Implications in Gynecological Applications

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Abstract *Leonurus japonicus*, a widely used medicinal herb in traditional Chinese medicine, plays an important role in the treatment of gynecological disorders. This study systematically reviews the variation in secondary metabolic traits of *L. japonicus* and explores their functional associations with gynecological efficacy. The major classes of secondary metabolites, including alkaloids, flavonoids, and phenolic acids, exhibit significant variability across different germplasms, environments, and developmental stages. These variations are closely regulated by complex biosynthetic pathways and gene expression networks. Pharmacological evidence indicates that these metabolites contribute to key therapeutic effects such as promoting blood circulation, regulating menstruation, and exerting anti-inflammatory and antioxidant activities. Furthermore, metabolomic analyses reveal strong correlations between specific compounds and clinical efficacy, highlighting the importance of metabolic profiling in quality evaluation. A case study comparing samples from different geographical origins further demonstrates that metabolic differences significantly influence therapeutic outcomes. Overall, this work provides a comprehensive framework for understanding the biochemical basis of *L. japonicus* efficacy and supports future applications in precision breeding and standardized utilization.
Keywords *Leonurus japonicus*; Secondary metabolites; Metabolic variation; Gynecological efficacy; Metabolomics

1 Introduction

Leonurus japonicus Houtt., commonly known as Chinese motherwort, has been esteemed for centuries in East Asian traditional medicine, particularly for its pivotal role in gynecological health. Its earliest documentation appears in the ancient Chinese pharmacopeia, Shennong Bencao Jing, where it was described as the “sacred medicine of gynecology” due to its efficacy in treating menstrual irregularities, postpartum hemorrhage, and other reproductive disorders (Shang et al., 2014; Wang et al., 2025). Over the past decades, *L. japonicus* has remained widely used in both traditional and modern clinical practice for conditions such as dysmenorrhea, amenorrhea, and blood stasis. This enduring popularity has driven extensive phytochemical and pharmacological research into its therapeutic mechanisms.

Central to the medicinal efficacy of *L. japonicus* is its rich repertoire of secondary metabolites-organic compounds essential for plant defense and ecological adaptation (Yeshi et al., 2022; Elshafie et al., 2023). These include alkaloids, flavonoids, phenolic acids, terpenoids, and other bioactive compounds, which exhibit diverse biological activities. Studies have shown that these metabolites regulate uterine contraction, exert anti-inflammatory effects, and protect against oxidative stress, thereby supporting their gynecological applications. In addition, environmental factors such as light, temperature, and soil conditions can influence the composition and accumulation of these compounds.

Despite significant progress in identifying over 280 compounds from *L. japonicus*, the biosynthetic pathways underlying their production remain incompletely understood. Recent genomic studies have revealed gene clusters associated with specialized metabolism and highlighted evolutionary mechanisms contributing to metabolite diversity (Li et al., 2023; Wang et al., 2024). Moreover, both genetic variation and environmental conditions can lead to substantial differences in metabolite content, which has important implications for medicinal quality and standardization. Multi-omics approaches are increasingly being used to elucidate the regulatory networks governing metabolite biosynthesis and accumulation.

Given this context, the present review aims to synthesize current knowledge on the variation in secondary metabolite traits of *L. japonicus* and explore their functional implications in gynecological applications. It focuses on the relationship between metabolic diversity and pharmacological efficacy, as well as the underlying genetic and environmental determinants. By integrating ethnobotanical knowledge with modern molecular research, this study seeks to provide a comprehensive framework for the standardized utilization and future development of *L. japonicus*.

2 Botanical Characteristics and Resource Distribution of *Leonurus japonicus*

2.1 Taxonomic status and morphological characteristics

Leonurus japonicus Houtt. is an herbaceous species in the family Lamiaceae, subfamily Lamioideae, and belongs to the small genus Leonurus, which comprises roughly 25 species distributed mainly across temperate to tropical Eurasia (Oak et al., 2021). Within the genus, *L. japonicus* is closely related to other medicinal taxa such as *L. cardiaca* and *L. sibiricus*, but differs in both chromosome number (2n = 20) and characteristic accumulation of leonurine, features that support its taxonomic separation and pharmacological distinctiveness (Yang et al., 2022). Floral and pollen micromorphology studies show that *L. japonicus* shares the typical Lamiaceae traits of zygomorphic, bilabiate corollas and bi-reticulate pollen exine, reinforcing its placement in Leonureae while also providing diagnostic features to distinguish it from congeners.

Morphologically, *L. japonicus* is an annual or biennial herb reaching up to 1-1.5 m, with square stems, opposite leaves, and verticillaster inflorescences typical of Lamiaceae (Rojas-Sandoval and Acevedo-Rodríguez, 2022). Leaves are palmately lobed and serrate, while the purple to pink bilabiate flowers bear abundant glandular and non-glandular trichomes that contribute to both defense and secretion of aromatic and medicinal compounds. In China, the plant flowers mainly from June to September and fruits from September to October, though in tropical regions it can flower throughout the year, reflecting notable phenological plasticity (Figure 1) (Huang et al., 2020).

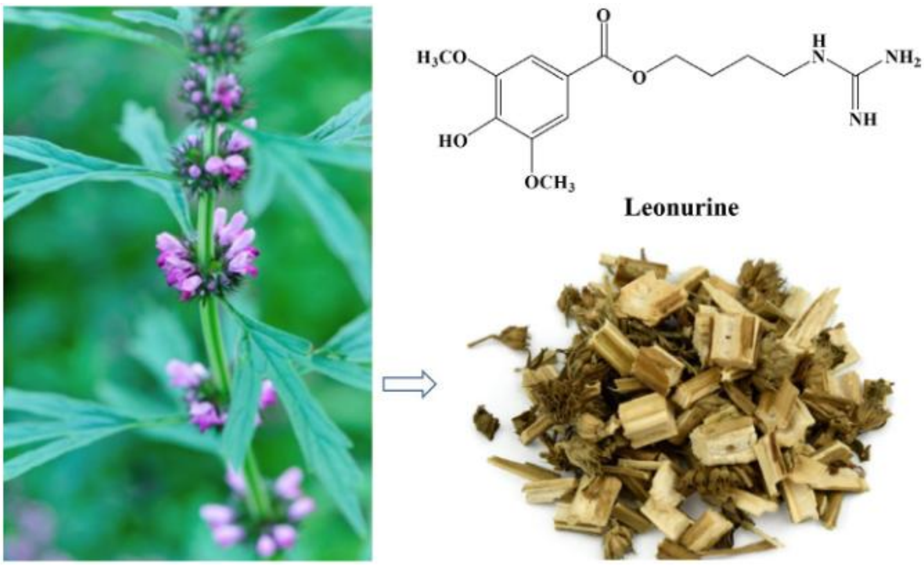

Figure 1 The photograph of *Leonurus japonicus* Houtt. and the structure of leonurine (Adopted from Huang et al., 2020)

2.2 Geographic distribution and ecological adaptability

Leonurus japonicus is native to East Asia but now shows a nearly cosmopolitan distribution, occurring in temperate and tropical regions of Asia, parts of Europe, and the Americas as an introduced or naturalized species (Hu et al., 2025). Within China, it is widely distributed from lowlands to montane areas, supporting its long history of use and large domestic demand as a medicinal resource. Habitat-suitability modeling indicates that its global and national centers of occurrence are concentrated in Southeast Asia, southern and eastern China, and extended regions such as South America and the Gulf of Mexico, underscoring its strong capacity for range expansion (Chen et al., 2024a).

Ecologically, *L. japonicus* prefers moist environments and is commonly found along field margins, riverbanks, disturbed ground, and grasslands from sea level up to about 3400 m (Rojas-Sandoval and Acevedo-Rodríguez, 2022). The species tolerates semi-shade but grows best in full sun and on a wide range of soils with pH 4-8, highlighting broad edaphic adaptability. Niche-modeling studies further reveal that precipitation in the warmest quarter and temperature in the coldest quarter are key climatic factors shaping its distribution, and future climate scenarios predict a northward and upward shift in suitable ranges, with expansion at higher latitudes (Wang et al., 2023a).

2.3 Genetic diversity and germplasm resources

Genetic studies based on complete chloroplast genomes and plastome hotspot regions have shown that *L. japonicus* in China possesses relatively low average nucleotide diversity ($\pi \approx 0.00029$) but is structured into four well-supported clades, reflecting historical divergence influenced by geological events such as the uplift of the Hengduan Mountains and Quaternary climate oscillations. Sliding-window analyses identified variable intergenic spacers (petN-psbM and rpl32-trnL) that serve as cost-effective markers for genotype discrimination and provide useful tools for monitoring population structure in germplasm collections (Wang et al., 2023b). AFLP and ISSR marker analyses across multiple accessions and provenances have also revealed rich polymorphism and clear clustering, confirming that substantial genetic variation persists despite localized bottlenecks (Chen et al., 2009; Wang, 2009).

At a broader phylogenetic scale, ITS-based analyses within Leonurus and comparative genomic work with L. sibiricus indicate that *L. japonicus* forms distinct genetic lineages, with chromosome-level genome assemblies now available to support fine-scale exploration of biosynthetic and adaptive traits (Yang et al., 2022; Arabova et al., 2025). Recent work combining DNA barcoding (ITS + plastid loci) with HPLC profiling has further shown that genetic groupings among different geographic origins correlate with variation in active ingredient content, although environmental factors also contribute significantly to metabolite differences (Figure 2) (Han et al., 2023; Hu et al., 2025). These findings highlight the importance of conserving diverse wild populations and developing genotype-informed germplasm banks to secure both genetic diversity and the spectrum of secondary metabolite phenotypes relevant to gynecological applications.

3 Types and Composition of Secondary Metabolites in *Leonurus japonicus*

Leonurus japonicus exhibits a chemically diverse profile with over one hundred secondary metabolites, including alkaloids, flavonoids, phenolic acids, and terpenoids, which collectively contribute to its gynecological effects such as uterotonic, anti-inflammatory, and antioxidant activities. Although pharmacopoeial standards often rely on single markers like leonurine, modern analyses reveal complex multi-component interactions influenced by plant origin and processing (Zhao et al., 2022; Han et al., 2023). Advanced chromatographic and metabolomic studies have further identified over 130 compounds, highlighting key groups such as alkaloids, flavonoids, and terpenoids as central to its therapeutic functions.

3.1 Alkaloids

Alkaloids are widely regarded as the primary signature metabolites of Leonurus, distinguishing *L. japonicus* from many other Lamiaceae that are dominated by terpenoids alone (Zhang et al., 2018; Li et al., 2023). Leonurine, a guanidine-type pseudoalkaloid, together with betaine-type alkaloids such as stachydrine and trigonelline, represents the major nitrogen-containing constituents quantified in pharmacopeial materials and in pharmacokinetic studies. Targeted LC-MS/MS analysis across different plant parts has confirmed these three molecules as the principal activity-related substances, with measurable stability and reproducible content suitable for use as quality markers (Zhao et al., 2022). Multi-omics comparison between *L. japonicus* (high-leonurine) and L. sibiricus (trace-leonurine) further shows that leonurine accumulation is species-specific and controlled by a specialized biosynthetic pathway, explaining why Chinese motherwort is particularly rich in this gynecological alkaloid.

Functionally, leonurine and stachydrine are strongly implicated in the uterine and cardiovascular actions that justify traditional indications such as regulation of menstruation, treatment of dysmenorrhea, and promotion of postpartum lochia discharge (Zhang et al., 2018). Ethnopharmacological and pharmacological syntheses emphasize that these alkaloids exert uterotonic effects, improve uterine blood flow, and modulate platelet aggregation and vascular tone, linking them directly to relief of blood-stasis-type gynecological syndromes and prevention of thrombotic complications in the puerperium (Shang et al., 2014; Zhang et al., 2023). Leonurine additionally exhibits cardioprotective, neuroprotective, and anti-oxidative activities, which may benefit women with comorbid cardiovascular risk or stress-related menstrual irregularities. The recent elucidation of leonurine biosynthesis-highlighting arginine decarboxylase, UDP-glucosyltransferase, and serine-carboxypeptidase-like acyltransferase as key enzymes-creates opportunities to breed or engineer high-leonurine lines, thereby optimizing uterotonic and cardioprotective potential in gynecological formulations.

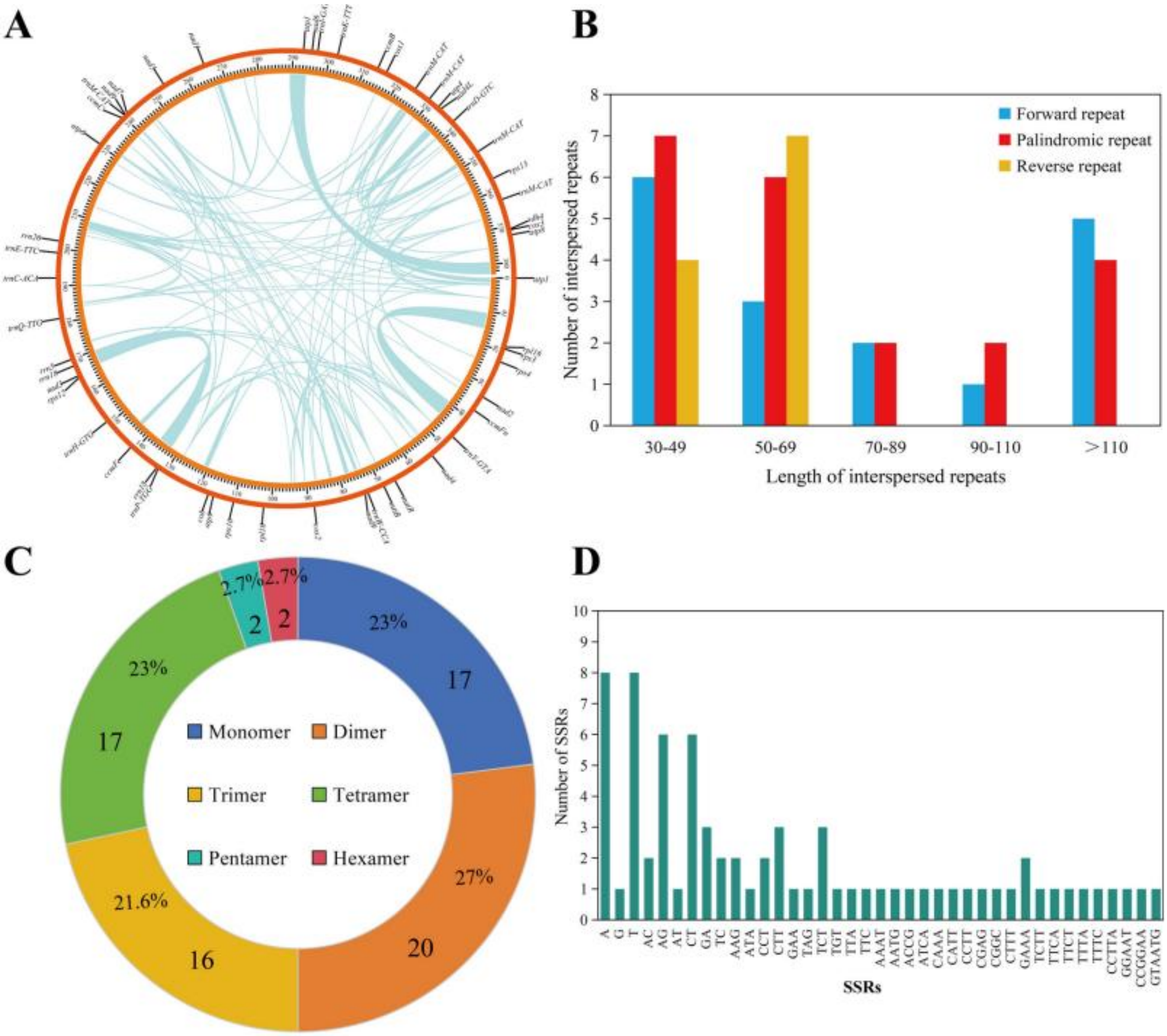

Figure 2 Repeat sequence analysis of the *L. japonicus* mt genome (Adopted from Hu et al., 2025)

Image caption: (A) The distribution of repeat sequences. The outer circle displays different genes. The blue inner arc indicates repeat sequences that are greater than or equal to 30 bp in length. (B) Histograms of lengths and classes of different DSRs. Different colors represent different types of DSRs. The x-axis and y-axis indicate the length and quantity of DSRs, respectively. (C) Proportion of different types of SSRs. Different colors represent different types of SSRs. (D) Statistical histograms of various SSRs. The x-axis displays different SSRs, while the y-axis shows the length of each SSR (Adopted from Hu et al., 2025)

3.2 Flavonoids and phenolic acids

Flavonoids and phenolic acids constitute a second dominant class of secondary metabolites in *L. japonicus*, widely distributed in aerial parts and readily enriched by polar extraction (Malave et al., 2020; Morais et al., 2023).

Quantitative assays show that ethanolic and hydroalcoholic extracts contain substantial amounts of total phenolics and flavonoids, with compounds such as chlorogenic, caffeic, caffeoylmalic, and ferulic acids, as well as rutin and quercetin, consistently identified in LC-MS analyses. Earlier isolation work from aerial parts yielded a panel of flavonoids including apigenin, luteolin, kaempferol, quercetin, and myricetin, alongside simple phenolic acids such as gallic and syringic acids, many of which display strong free-radical-scavenging activity in DPPH assays (Qu et al., 2006). More recent metabolomic studies on Leonurus spp. confirm that phenylpropanoids-including rare caffeoylglucaric acids-and classical flavonoids are quantitatively dominant in the phenolic fraction, emphasizing their central contribution to the plant's antioxidant capacity (Olennikov and Chirikova, 2016).

These phenolic constituents are closely linked to activities relevant for gynecological health, especially anti-oxidative, anti-inflammatory, vascular-protective, and tissue-repair effects that accompany uterine and pelvic pathologies. Antioxidant testing consistently demonstrates that phenolic-rich partitions (e.g., ethyl acetate fractions) and individual flavonoids such as luteolin, kaempferol, and quercetin exhibit higher radical-scavenging power than reference antioxidants, supporting their role in limiting oxidative damage during menstrual pain or postpartum recovery (Qu et al., 2006). In a wound-healing model, hydroalcoholic extracts with higher flavonoid content accelerated inflammatory resolution, wound contraction, and collagen synthesis, suggesting that polyphenols from *L. japonicus* can facilitate repair of reproductive-tract and perineal injuries associated with childbirth or gynecological surgery. Phenolic acids such as chlorogenic and caffeic acid, together with rutin and related flavonoid glycosides, also correlate with anti-inflammatory, vasodilatory, and enzyme-inhibitory activities in Leonurus species, providing mechanistic support for their use in relieving pelvic congestion, dysmenorrhea, and microvascular complications associated with gynecological disorders (Angeloni et al., 2021).

3.3 Volatile oils and other bioactive compounds

Beyond alkaloids and phenolics, *L. japonicus* produces essential oils and a suite of terpenoid and minor constituents that add further pharmacological dimensions, particularly antimicrobial and anti-inflammatory effects relevant to gynecological infections and postpartum care. Hydrodistillation of the herb (Yimucao) and fruits (Chongweizi) reveals marked variation in essential-oil profiles between parts: herb oil is dominated by sesquiterpenes and diterpenes, with phytone, phytol, caryophyllene oxide, and β-caryophyllene as major components, whereas fruit oil is richer in aliphatic hydrocarbons and bornyl acetate. These compositional differences translate into distinct bioactivities; Yimucao oil shows broad-spectrum activity against Gram-positive bacteria, while Chongweizi oil is largely inactive, underscoring the herb's particular value for managing genital and puerperal infections in traditional practice (Xiong et al., 2013). The predominance of β-caryophyllene, a sesquiterpene with known anti-inflammatory and analgesic potential, further suggests synergism with uterotonic alkaloids and phenolics in alleviating pelvic pain and inflammatory gynecological conditions.

In addition to essential oils, numerous labdane-type diterpenoids, ionone derivatives, terpenoid acids, and triterpenes have been isolated from the aerial parts of *L. japonicus*, many of them newly characterized in the last decade (Zhong et al., 2015; Wei et al., 2023). Several labdane diterpenes, including novel epimeric pairs, significantly inhibit nitric oxide production and pro-inflammatory cytokine release in LPS-stimulated macrophages, partly by blocking NF-κB signaling, thereby providing a non-alkaloid anti-inflammatory axis that may contribute to relief of uterine inflammation, endometritis, or pelvic inflammatory disease. Other terpenoids such as leojaponic acids A and B and triterpenes like ursolic acid exhibit enzyme-inhibitory and cytoprotective activities, which, together with minor phenylethanoid glycosides and iridoids documented in the genus, broaden the spectrum of biological actions relevant to women's health (Wu et al., 2016; Angeloni et al., 2021). Collectively, the volatile and terpenoid fractions complement the hormone-modulating and hemodynamic actions of alkaloids with antimicrobial, anti-inflammatory, and tissue-protective effects, forming a multi-target phytochemical ensemble well-suited to complex gynecological syndromes that involve infection, inflammation, pain, and impaired uterine involution.

4 Variation in Secondary Metabolic Traits and Influencing Factors

4.1 Metabolic differences among germplasms and varieties

Leonurus japonicus exhibits substantial intraspecific variation in secondary-metabolite traits that is closely tied to its genetic diversity across populations. Phylogenetic work based on concatenated plastid and nuclear markers has divided Chinese germplasm into four deeply divergent clades, with divergence events linked to geological uplift and Quaternary climate shifts (Wang et al., 2023a). This population structure provides a genomic framework for chemotype formation, because nucleotide diversity "hotspots" and clade differentiation are expected to underlie differences in biosynthetic capacity and regulatory networks influencing alkaloids, flavonoids, and terpenoids. At the same time, comparative genomics within the genus shows that leonurine accumulation is species-specific, driven by duplication and neofunctionalization of a UGT-SCPL gene cluster in *L. japonicus* but not in the low-leonurine species L. sibiricus, emphasizing how relatively recent gene-level changes can create marked metabolic contrasts between otherwise related lineages (Li et al., 2023).

Chemotaxonomic analysis of accessions from multiple provenances confirms that germplasms differ not only in overall metabolite content but also in the relative proportions of leonurine and other active markers important for gynecological efficacy (Han et al., 2023). High-performance liquid chromatography combined with multivariate statistics has resolved origin-specific clusters, in which some populations consistently show higher levels of key uterotonic and cardioprotective constituents than others, even under similar analytical conditions. These chemotype differences likely translate into variable clinical potency for indications such as regulating menstruation or promoting postpartum uterine involution, and they underscore the need to match germplasm to pharmacopoeial standards when developing standardized modern preparations. Integrating genome information with metabolite profiling-linking specific clades or alleles to leonurine and phenolic contents-offers a path toward breeding elite lines with optimized secondary-metabolite profiles for gynecological applications.

4.2 Regulation of metabolism by environmental factors

Environmental conditions strongly modulate secondary-metabolite accumulation in medicinal plants, and *L. japonicus* is no exception. A multi-origin study combining phylogenetics and chemical analysis showed that differences in active-ingredient profiles among regions could not be explained by genetics alone, implying a major contribution of local climate and edaphic conditions to observed metabolic variation (Han et al., 2023). In parallel, ecological modeling at the species level has identified precipitation patterns and temperature regimes-especially precipitation of the warmest quarter and minimum temperature of the coldest month-as key determinants of ecological suitability, with highly suitable habitats tending to support plants with higher expression of medicinal markers (Chen et al., 2024a). These findings suggest that water availability and thermal stress shape secondary metabolism in the field, for example by influencing pathways for alkaloids and phenylpropanoids that underpin gynecological indications such as hemostasis and uterine activation.

More broadly, reviews on plant secondary metabolism under abiotic stress demonstrate that light intensity and spectrum, temperature, soil moisture, nutrient status, and salinity can all shift both the quantity and profile of secondary metabolites, often through transcriptional regulation of biosynthetic genes (Jan et al., 2021). Even modest variation in a single factor-such as soil water or fertility-can significantly alter the levels of phenolics, terpenes, or alkaloids while other factors remain constant, indicating that cultivation practices for *L. japonicus* must carefully manage irrigation, fertilization, and shading to maintain consistent chemical quality (Yang et al., 2018). Climate-change-oriented syntheses further highlight that elevated temperature, drought, and changing CO_2 can either enhance or suppress specific metabolite classes, implying that future field production of motherwort may drift in chemotype unless adaptive agronomic strategies are implemented to stabilize uterotonic and anti-inflammatory components critical for gynecological efficacy (Qaderi et al., 2023).

4.3 Effects of developmental stages and harvesting time

Ontogeny exerts a powerful influence on secondary metabolism, and developmental regulation needs to be considered when defining optimal harvest windows for gynecological use of *L. japonicus*. General analyses of medicinal plants show that biosynthesis and storage of key secondary metabolites are tightly linked to organ differentiation, flowering, and reproductive investment, resulting in stage-specific peaks of alkaloids, flavonoids, and volatile terpenoids (Li et al., 2020a). During early vegetative and pre-flowering phases, carbon allocation often favors phenylpropanoids and simple flavonoids that provide oxidative and UV protection, whereas later reproductive stages can shift metabolism toward terpenoid volatiles and lignified phenolics, altering the balance of compounds relevant to uterine and hemostatic actions. For *L. japonicus*, such ontogenic patterns imply that aerial parts harvested at different phenological stages (pre-flowering herb versus fruiting tops) may differ markedly in leonurine, flavonoid, and essential-oil content, potentially leading to variable gynecological outcomes if not standardized.

Evidence from related medicinal species illustrates how tightly secondary-metabolite dynamics can track developmental transitions and supports extrapolation to motherwort. In Sophora japonica, UHPLC-based metabolomics across five flower-maturity stages revealed pronounced shifts in 331 metabolites, with flavonoids and phenolic acids showing the strongest variation and early buds being richest in pharmacologically valued flavonoids suited for medicinal extraction (Wang et al., 2022a). Similarly, integrative omics of Lonicera japonica flowers demonstrated that simple phenylpropanoids and flavonoids accumulate predominantly at early stages, while terpenoid-backbone metabolites increase later, indicating a developmental switch in pathway dominance (Yang et al., 2019). Together with broader reviews on developmental control of medicinal plant metabolites, these results suggest that to maximize uterotonic alkaloid and antioxidant polyphenol content for gynecological formulations, *L. japonicus* should be harvested at carefully defined stages-likely around full flowering of the herb and specific maturity of the fruits-supported by stage-resolved metabolomic profiling and linked to traditional experiential criteria (Li et al., 2020b).

5 Biosynthetic Pathways and Molecular Regulation of Secondary Metabolites

Leonurus japonicus accumulates structurally diverse secondary metabolites-including leonurine, labdane-type diterpenoids and flavonoids-whose biosynthesis relies on conserved primary pathways such as shikimate, phenylpropanoid and terpenoid backbones that are broadly shared across angiosperms (Jan et al., 2021; Zhan et al., 2022). In many medicinal plants, the shikimate pathway supplies aromatic amino acids, which in turn feed into phenylpropanoid and flavonoid biosynthesis, creating the scaffold for tissue-specific and stress-responsive metabolite profiles relevant to pharmacological activity. Terpenoid and diterpenoid scaffolds in *L. japonicus* derive from plastidial and cytosolic isoprenoid pathways, with subsequent tailoring by cytochrome P450s, glycosyltransferases and acyltransferases producing lineage-specific compounds such as spiro-labdane diterpenoids and leonurine that underlie its distinctive gynecological uses. Integration of genomics and metabolomics in Leonurus indicates that diversification of specialized enzymes and gene clusters not only shapes total metabolite output but also species-specific differences between *L. japonicus* and low-leonurine relatives, highlighting an evolutionary tuning of biosynthetic capacity (Li et al., 2023).

5.1 Key metabolic pathways and enzymatic reactions

Multi-omics reconstruction of leonurine biosynthesis shows that this key guanidine-containing alkaloid arises from arginine via arginine decarboxylase (ADC), followed by uridine diphosphate glucosyltransferase (UGT)-mediated glycosylation and serine carboxypeptidase-like (SCPL) acyltransferase-catalyzed acylation, defining a concise route from primary nitrogen metabolism to a pharmacologically active secondary metabolite (Li et al., 2023). Comparative genomics between *L. japonicus* and L. sibiricus reveals that expansion and neofunctionalization of UGT-SCPL gene clusters in *L. japonicus* are central to its high leonurine content, illustrating how small changes in enzyme complement can dramatically shift the quantitative profile of medicinally important metabolites.

Labdane-related diterpenoids, including spiro-9,13-epoxy-labdane structures abundant in *L. japonicus*, are formed by a pairwise action of class II and class I diterpene synthases acting on geranylgeranyl diphosphate to generate peregrinol diphosphate and then epoxy-bridged labdane skeletons (Wang et al., 2022b). Functional characterization of six *L. japonicus* diTPSs indicates that LjTPS3 supplies the C-9-hydroxylated intermediate, whereas LjTPS6 produces a mixture of labdane products; mutational analysis of a single active-site residue in LjTPS6 can shift product specificity toward a single 9,13-epoxy-labdane epimer, underscoring how subtle protein changes reprogram diterpenoid profiles.

5.2 Regulatory networks of related genes and transcription factors

Genome-level analyses of *L. japonicus* identify expanded gene families in specialized metabolism, particularly diterpenoid biosynthesis, suggesting that duplication and diversification of pathway genes provides a genomic substrate for regulatory rewiring of secondary metabolite output (Wang et al., 2024). In Leonurus WRKY transcription factor (TF) families, drought-responsive members (e.g., LjWRKY1, 4, 23, 44) show strong induction or repression under stress, and differentially expressed genes under drought are enriched in plant hormone signaling, MAPK cascades and secondary metabolite biosynthesis, implying a coordinated TF-centered network that links environmental cues to metabolite pathways (Guo et al., 2025).

Across plant species, transcription factors from WRKY, MYB, AP2/ERF, bHLH, bZIP and NAC families act as master regulators of secondary metabolism by binding cis-elements in promoters of biosynthetic genes and modulating their transcription in response to biotic and abiotic stimuli (Jan et al., 2021; Rabeh et al., 2025). Stress-inducible TFs often converge on precursor-producing steps such as the shikimate and phenylpropanoid pathways, creating regulatory nodes where environmental signals can adjust flux toward flavonoids, alkaloids or terpenoids; these principles are likely conserved in *L. japonicus* and underpin stress- and tissue-dependent variability in leonurine and labdane diterpenoids (Zhan et al., 2022).

5.3 Advances in molecular markers and metabolic regulation

The chromosome-level genome of *L. japonicus*, with over 22,000 annotated genes and clear expansion of specialized-metabolism families, provides a foundational resource for designing molecular markers targeting loci involved in diterpenoid and alkaloid biosynthesis and for associating allelic variation with chemotype differences among germplasm (Wang et al., 2024). Population-level work correlating ITS and plastid markers with inter-origin variation in active components already shows that genetic divergence only partially explains metabolite differences, emphasizing the need for pathway-anchored markers that can directly track biosynthetic capacity in breeding for stable gynecological quality traits (Han et al., 2023).

Multi-omics approaches in related medicinal species illustrate how integrating transcriptomics, metabolomics and co-expression network analysis can reveal key control points and candidate regulators for flavonoid and other secondary-metabolite pathways, offering a template for similar systems-level dissection in *L. japonicus* (Yang et al., 2019; Chen et al., 2024b). In such frameworks, hub TFs (including WRKYs) and tailoring enzymes like UGTs emerge as central levers of metabolic regulation; manipulating these nodes by marker-assisted selection, transgenic or genome-editing strategies could rationally enhance leonurine and labdane-diterpenoid profiles that contribute to uterotonic, hemostatic and anti-inflammatory effects in gynecological applications (Li et al., 2023; Guo et al., 2025).

6 Pharmacological Effects of *Leonurus japonicus* in Gynecological Disorders

Leonurus japonicus, known as a “sacred medicine of gynecology,” has long been used to treat menstrual disorders and postpartum conditions. Its major secondary metabolites, including alkaloids, flavonoids, and terpenoids, exert multi-target effects on circulation, inflammation, and endocrine regulation, supporting its therapeutic role (Shang et al., 2014; Li et al., 2019). Modern studies indicate that these compounds act synergistically through multiple pathways, providing a mechanistic basis for its traditional functions such as promoting blood circulation and alleviating gynecological disorders.

6.1 Mechanisms of promoting blood circulation and regulating menstruation

Animal models of trauma-induced blood stasis demonstrate that *L. japonicus* extracts significantly reduce plasma viscosity, platelet aggregation, fibrinogen, thromboxane B_2, and plasminogen activator inhibitor-1, while increasing antithrombin III and tissue-type plasminogen activator, indicating coordinated antithrombotic and profibrinolytic effects that improve microcirculation (Zhang et al., 2023). In the same model, extract treatment ameliorates local tissue injury and normalizes hemodynamic indices, supporting its use for pelvic “blood stasis” conditions that underlie dysmenorrhea and amenorrhea in traditional gynecology (Miao et al., 2019).

Leonurine and stachydrine also display vasodilatory, anti-platelet, and angiogenic activities, providing compound-level mechanisms for improved uterine blood supply and endometrial repair (Liao et al., 2023; Shi et al., 2022). Total alkaloid fractions from *L. japonicus* promote endothelial cell proliferation, migration, and tube formation via SRC/MEK/ERK signaling and accelerate wound healing *in vivo*, suggesting that enhanced neovascularization may contribute to the resolution of ischemic or stagnant uterine states associated with irregular menstruation and postpartum recovery (Li et al., 2019).

6.2 Anti-inflammatory and antioxidant activities

Motherwort total alkaloids attenuate bacteria-induced endometritis in rats, where treatment reduces inflammatory mediator overproduction and promotes endometrial repair through suppression of the PI3K/AKT/NF-κB axis and downstream cytokines (Ou et al., 2025). *in vitro*, these alkaloids inhibit lipopolysaccharide-triggered inflammatory responses in macrophages and human endometrial epithelial cells, highlighting a direct action on uterine immune-inflammatory microenvironments that are central to chronic endometrial pathology and infertility.

Broader pharmacological profiling shows that leonurine and stachydrine possess prominent anti-inflammatory and antioxidant properties, including inhibition of oxidative stress, modulation of NF-κB signaling, and reduction of tissue injury in cardiovascular and neuronal models (Liao et al., 2023; Wang et al., 2025). Crude *L. japonicus* extracts similarly downregulate TNF-α, IL-6, and IL-8 while enhancing anti-inflammatory IL-10 and antioxidant systems in trauma blood-stasis rats, indicating that both purified metabolites and complex mixtures can temper systemic and local inflammation relevant to pelvic pain, dysmenorrhea, and inflammatory gynecological disorders (Miao et al., 2019; Zhang et al., 2023).

6.3 Effects on uterine contraction and endocrine regulation

Classical and modern data converge to show that *L. japonicus* has marked effects on the uterus, including the ability to stimulate uterine smooth muscle and thereby assist in treating postpartum hemorrhage caused by uterine inertia (Shang et al., 2014). Bioassay-guided isolation has revealed that specific cyclopeptides and alkaloids, including leonurine, enhance contraction of rat uterine strips, while flavonoid glycosides exert the opposite, relaxing effect, explaining the herb’s clinically observed bidirectional regulation of uterine activity in dysmenorrhea versus postpartum bleeding. More recently, coumarins isolated from *L. japonicus* were found to have similarly opposite effects on uterine smooth muscle: bergapten promotes contraction by increasing intracellular Ca^{2+} via L-type Ca^{2+} channels and α-adrenergic receptors, whereas osthole, an α-receptor antagonist, reduces Ca^{2+} influx and relaxes oxytocin-induced contractions (Fan et al., 2024). Beyond direct myometrial actions, network pharmacology analyses indicate that multiple motherwort components target endocrine-related molecules such as ESR1, AR, AKT1, and PPARG, suggesting integrated regulation of steroid hormone signaling in menstrual disorders (Wang et al., 2020).

Endocrine modulation by *L. japonicus* extends to ovarian steroidogenesis, where luteolin and luteolin-7-methylether suppress aromatase-mediated estrogen biosynthesis in human granulosa cells by inhibiting TPL2-MKK3/6-p38-CREB signaling (Shi et al., 2024). Luteolin-7-methylether (XLY29) decreases estradiol production in granulosa-like cells and downregulates aromatase promoter I.3/II without directly inhibiting catalytic activity, and *in vivo* lowers serum estradiol and alters estrous cycling in mice, suggesting potential applications in estrogen-excess conditions such as polycystic ovary syndrome (Du et al., 2020). A network pharmacology study focused on menstrual disorders further shows that 29 bioactive compounds from motherwort share core targets in endocrine, vascular, and inflammatory pathways, with hub genes including AKT1, PTGS2, ESR1, AR, and PPARG. Molecular docking indicates that many of these metabolites bind strongly to estrogen receptor and androgen receptor, providing a mechanistic bridge between traditional indications of “regulating menstruation” and modern concepts of hypothalamic-pituitary-ovarian axis and peripheral receptor modulation in gynecological endocrine disorders.

7 Functional Associations Between Secondary Metabolic Traits and Gynecological Effects

7.1 Correlation between key active components and pharmacological efficacy

Chemical-pharmacological correlation studies consistently identify leonurine, stachydrine and trigonelline as core active alkaloids whose plasma exposure and tissue distribution parallel the traditional gynecological indications of *Leonurus japonicus* (Zhao et al., 2022). Network pharmacology and pharmacokinetic analyses show that these alkaloids act on targets involved in vascular regulation, inflammation and endocrine pathways, linking their presence in different plant parts to effects on menstrual disorders, postpartum recovery and blood-stasis syndromes (Wang et al., 2020).

At the single-compound level, leonurine displays pleiotropic cardiovascular, anti-oxidant and anti-inflammatory actions, furnishing mechanistic support for motherwort's use in conditions characterized by pelvic ischemia, pain and microvascular dysfunction (Li et al., 2019). Other secondary metabolites, including labdane diterpenoids and flavonoids, contribute anti-platelet, anti-inflammatory and hormone-modulating activities, suggesting that distinct metabolite classes collectively underpin the multi-target efficacy observed in obstetrical and gynecological practice (Xiao et al., 2017; Zhang et al., 2018).

7.2 Impact of metabolic variation on therapeutic stability

Comparative analyses of *L. japonicus* from different geographic origins reveal significant intergroup differences in active-ingredient profiles, despite relatively modest genetic divergence, indicating that environmental factors and cultivation conditions strongly influence secondary metabolite composition (Han et al., 2023). Such chemotypic variation implies that the content of key alkaloids or diterpenoids may fluctuate among accessions, potentially leading to inconsistent clinical responses when crude drugs are sourced without standardized quality control (Wang et al., 2023b).

A systematic Q-marker study further demonstrates that leonurine, stachydrine and trigonelline differ in stability during storage, with time-dependent declines that are substantial enough to affect pharmacologically effective doses if not controlled (Zhao et al., 2022). By establishing content limits and stability windows for these markers, the work directly links metabolic degradation dynamics to therapeutic reliability, providing a quantitative framework for ensuring consistent gynecological efficacy in commercial preparations (Wang et al., 2024).

7.3 Functional association studies based on metabolomics

Integrated metabolomics and network pharmacology applied to leonurine show that changes in circulating metabolites map to pathways in glycerophospholipid, linoleic acid, tryptophan and glutamate metabolism, which are central to oxidative stress, inflammation and energy homeostasis (Rong et al., 2022). Regulation of these metabolic networks aligns with leonurine's protective effects in cardiovascular and metabolic models, reinforcing the idea that modulation of systemic metabolism is a key route through which *L. japonicus* components support uterine and pelvic vascular health in gynecological disorders (Li et al., 2019).

In parallel, metabolomic and multivariate analyses of whole-plant extracts identify dozens of absorbed prototypes and biotransformation products whose patterns differ across plant parts, yet cluster with gynecologically relevant targets in network models (Zhao et al., 2022). These association studies suggest that specific metabolite signatures-rather than single compounds alone-correlate with anti-inflammatory, endocrine-modulating and pro-circulatory effects, pointing toward metabolomics-driven definition of chemotypes optimized for menstrual regulation, endometrial repair or estrogen-related conditions (Shi et al., 2024).

8 Case Study: Comparative Analysis of Metabolic Variation and Gynecological Efficacy of *Leonurus japonicus* from Different Origins

8.1 Comparative analysis of chemical composition among different geographical origins

Leonurus japonicus exhibits pronounced intraspecific chemical variation, reflecting both genetic divergence and environmental heterogeneity across its distribution range. Multi-locus barcoding combined with HPLC profiling has shown that accessions from different origins cluster into distinct genetic lineages, and these clades partially parallel differences in contents of key alkaloids such as leonurine and stachydrine (Figure 3) (Han et al., 2023).

The integrated analysis indicates that metabolite divergence cannot be explained by genetics alone, and that ecological variables-particularly climate and edaphic conditions-shape local chemotypes, likely through modulation of stress-responsive and specialized-metabolism gene networks. Broad-scale ecological modeling further supports this view: areas with higher climatic suitability for *L. japonicus* tend to harbor plants with more favorable profiles of medicinal marker compounds, linking habitat quality to metabolite accumulation (Chen et al., 2024a).

High-resolution metabolomics has revealed that beyond a few alkaloid markers, the *L. japonicus* metabolome encompasses extensive diversity in flavonoids, phenylethanoid glycosides, terpenoids, and fatty-acid esters, with many of these constituents varying quantitatively among origins (Garran et al., 2019). Comparative surveys suggest that some regions produce material enriched in alkaloids and flavonoids, whereas others are relatively richer in terpenoid scaffolds, implying origin-specific pharmacological tendencies within the same species. Large-scale distribution modeling predicts a future poleward expansion and reshaping of suitable areas under climate change, raising the prospect that both the geographic supply and typical metabolite profiles of medicinal *L. japonicus* will shift over time (Chen et al., 2024b). Together, these data underscore that “origin” encodes a complex combination of genetic background, local environment, and resulting metabolite pattern, with direct relevance to quality evaluation.

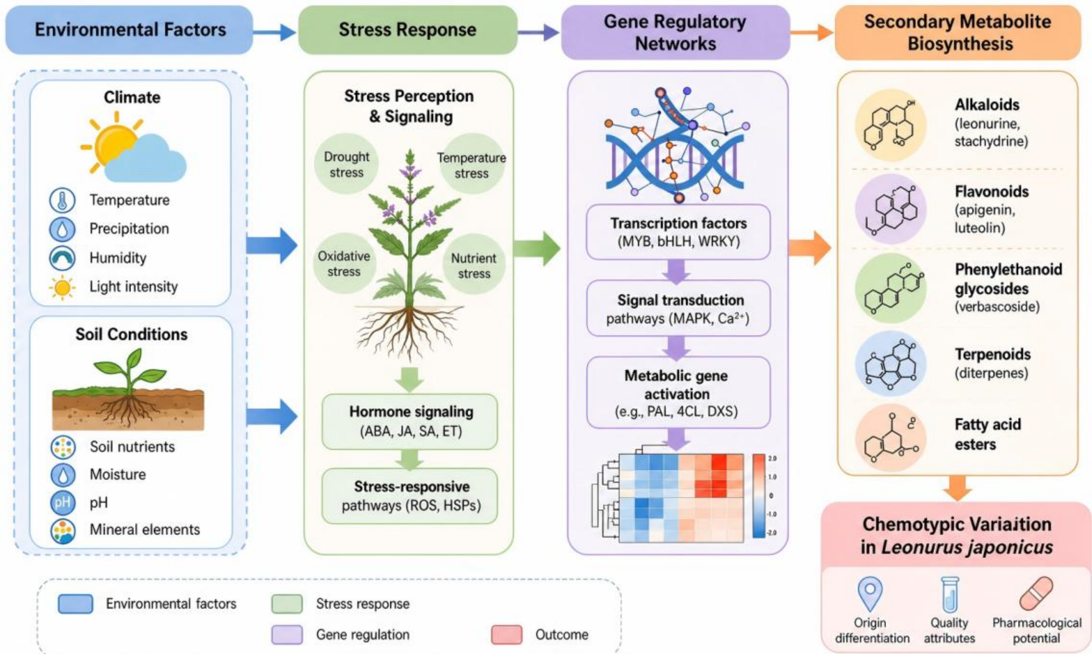

Figure 3 Conceptual model illustrating how environmental factors (climate and soil conditions) influence stress-responsive pathways and specialized metabolism gene networks, ultimately shaping chemotypic variation in *Leonurus japonicus* (Adopted from Han et al., 2023)

8.2 Pharmacodynamic evaluation (*in vitro* and *in vivo* models)

Pharmacodynamic studies integrating chemical profiling with bioassays have begun to clarify how origin-linked metabolite differences translate into gynecologically relevant activities. Total alkaloid fractions, dominated by leonurine-type and related bases, promote angiogenesis, endothelial migration, and collagen deposition via SRC-MEK-ERK signaling, supporting tissue repair after uterine or perineal injury (Shi et al., 2022). Parallel work in trauma-induced blood-stasis models demonstrates that whole-plant extracts rich in alkaloids and flavonoids improve hemodynamics, decrease platelet aggregation, and modulate vasoactive mediators, providing mechanistic support for traditional indications of “activating blood circulation” in dysmenorrhea and postpartum conditions (Zhang et al., 2023). Because the abundance of these bioactive constituents is origin-dependent, extracts from alkaloid-rich regions are expected to exert stronger pro-angiogenic and hemorheologic effects at equivalent doses (Han et al., 2023).

Gynecological inflammation models offer additional insight into how metabolite patterns shape efficacy. Motherwort total alkaloids significantly ameliorate bacteria-induced endometritis in rats, reducing inflammatory mediator overproduction and promoting endometrial repair through regulation of PI3K-AKT-NF-κB signaling (Ou et al., 2025). In parallel, combination formulas containing *L. japonicus* with other herbs show robust efficacy against bovine and rodent endometritis, lowering uterine bacterial load and inflammatory scores while improving oxidative status, consistent with a multi-component, multi-target anti-inflammatory mode of action (Tan et al., 2025). In endocrine-related disorders, *L. japonicus*-derived flavonoids such as luteolin and luteolin-7-methylether inhibit aromatase-mediated estrogen biosynthesis in granulosa cells and alleviate polycystic ovary syndrome (PCOS) phenotypes in mice, implicating origin-dependent flavonoid content in modulation of ovarian steroidogenesis (Du et al., 2020; Shi et al., 2024). Collectively, these *in vitro* and *in vivo* data indicate that variation in alkaloid and flavonoid levels across origins is likely to produce distinct profiles of uterotonic, anti-inflammatory, vascular, and endocrine activities.

8.3 Validation of correlations between metabolic profiles and clinical efficacy

Establishing robust links between origin-specific metabolite signatures and clinical gynecological outcomes requires coordinated chemical, pharmacokinetic, and clinical data. Multi-marker quality control work has identified leonurine, stachydrine, and trigonelline as quantitative “Q-markers” that are both pharmacodynamically relevant and sufficiently stable for routine measurement, enabling standardized comparison of materials from different regions and plant parts (Zhao et al., 2022). Broad metabolomic and genomic analyses demonstrate that *L. japonicus* accessions with higher expression of leonurine-biosynthesis genes accumulate more leonurine in aerial tissues, whereas closely related Leonurus species lacking these pathway innovations are essentially leonurine-free, highlighting a genetic basis for inter- and intra-specific differences in clinically important alkaloids. Integrating such markers with origin information allows retrospective correlation of clinical preparations-long valued for treating menoxenia, dysmenorrhea, amenorrhea, postpartum hemorrhage, and lochial disorders-with defined ranges of bioactive constituents (Shang et al., 2014; Miao et al., 2019).

Translational studies in gynecologically relevant models provide a bridge between chemistry and real-world therapeutic outcomes. Leonurine isolated from *L. japonicus* exhibits potent antimycobacterial activity against Mycobacterium tuberculosis and reduces mycobacterial load in a rat model of genital tuberculosis, directly linking an origin- and pathway-dependent alkaloid to improved uterine infection control (Gan et al., 2019). Similarly, motherwort-based formulations and extracts rich in leonurine and related secondary metabolites alleviate obstetric and gynecologic conditions such as postpartum hemorrhage, irregular menstruation, and dysmenorrhea in clinical and preclinical settings, in line with their vascular, uterotonic, anti-inflammatory, and endocrine-modulating activities (Wen et al., 2019). As ecological modeling now connects climatic suitability and gene expression with medicinal compound accumulation, origin-informed cultivation strategies can be used prospectively to produce chemotypes optimized for specific gynecologic indications, creating a feedback loop in which metabolic profiling, pharmacodynamics, and clinical efficacy mutually refine quality standards for *L. japonicus*-based therapies.

9 Conclusion and Future Perspectives

Research on *Leonurus japonicus* has firmly established it as a key medicinal plant in gynecology, with its therapeutic efficacy closely linked to a chemically diverse and dynamic secondary metabolite system. The plant contains abundant alkaloids, diterpenoids, and flavonoids, among which leonurine, stachydrine, and trigonelline are considered core bioactive compounds. These metabolites not only contribute to traditional indications such as regulating menstruation and promoting blood circulation but also serve as important quality markers connecting phytochemical variation with clinical consistency. Advances in chromatographic and metabolomic analyses have revealed substantial intra- and interspecific variation in these compounds, highlighting leonurine as a species-specific chemical signature distinguishing *L. japonicus* from related taxa such as L. sibiricus. More importantly, the integration of genomics, transcriptomics, metabolomics, and enzymology has enabled reconstruction of the leonurine biosynthetic pathway, identifying key enzymes such as arginine decarboxylase (ADC), UDP-glycosyltransferases (UGTs), and serine carboxypeptidase-like proteins (SCPLs). Gene duplication and functional divergence within these pathways provide a mechanistic explanation for the high accumulation of leonurine in *L. japonicus*, thereby directly linking genetic architecture with pharmacologically relevant alkaloid traits.

At the functional level, the diverse metabolite profile of *L. japonicus* underpins its multi-target pharmacological activities, particularly in gynecological contexts. Total alkaloid fractions and enriched extracts have been shown to promote angiogenesis, accelerate tissue repair, and facilitate endometrial recovery through signaling pathways such as SRC/MEK/ERK and PI3K/AKT/NF-κB, supporting its traditional application in postpartum rehabilitation and inflammatory uterine conditions. Systems pharmacology approaches further demonstrate that multiple metabolite classes collectively modulate endocrine, vascular, and inflammatory networks, with hub targets including AKT1, ESR1, and PTGS2 serving as key regulatory nodes. Flavonoids such as luteolin and luteolin-7-methylether provide an additional layer of endocrine regulation by suppressing aromatase-mediated estrogen biosynthesis via pathways such as p38 MAPK/AKT and TPL2-p38-CREB, suggesting therapeutic potential in estrogen-related disorders including polycystic ovary syndrome. Meanwhile, labdane diterpenoids, particularly those localized in glandular trichomes, exhibit anti-inflammatory, antithrombotic, and anti-proliferative activities, expanding the spectrum of bioactive constituents involved in regulating uterine blood flow, thrombosis, and pelvic inflammation. Together, these findings support a multi-component, multi-target mode of action that bridges traditional concepts with modern molecular pharmacology.

Despite significant progress, important challenges remain in fully elucidating the relationship between metabolite diversity and gynecological efficacy. Existing studies are often fragmented across different extract types, plant tissues, developmental stages, and geographical origins, with relatively few investigations systematically linking natural variation in metabolite profiles to standardized clinical outcomes. Although genetic and environmental factors have been shown to influence metabolite composition, the specific ecological drivers and agronomic strategies for optimizing key bioactive compounds remain insufficiently defined. Furthermore, while network pharmacology and *in vitro* or animal studies provide valuable mechanistic insights, rigorously designed clinical trials that incorporate chemical quality and genetic background are still lacking. The expansion of genomic resources, including chromosome-level assemblies and organelle genomes, offers new opportunities for integrative research; however, comprehensive genotype-metabolite-phenotype association studies remain in their early stages. Future research should prioritize multi-omics integration across diverse germplasm to develop predictive models linking genome, environment, and metabolite traits to gynecological functions. Such efforts, combined with precision breeding strategies and improved quality control frameworks that incorporate multi-component fingerprints and pharmacokinetic data, will be essential for advancing the standardized, safe, and evidence-based application of *L. japonicus* in modern obstetrics and gynecology.

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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.

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