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,