Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2026, Volume 6, Issue 1, Page No: 22-30

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Spatial Evidence Changes Biosynthetic Inference: Integrating Mass-Spectrometry Imaging, Spatial Transcriptomics, Cell-Type Identity, and Tissue Anatomy in Medicinal-Plant Pathway Reconstruction

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Received: 24 October 2025; **Revised:** 18 January 2026; **Accepted:** 21 January 2026

ABSTRACT

Reconstructing medicinal-plant biosynthetic pathways increasingly requires more than identifying enzymes, pathway genes, and candidate regulatory relationships. Conventional genomic, transcriptomic, biochemical, and heterologous-reconstruction approaches can establish pathway membership and reaction competence while leaving unresolved where pathway steps occur within intact tissues, whether intermediates move between cell types, and whether metabolite accumulation identifies synthesis, transport, or storage. This article develops a proposed spatial evidence-integration model that treats mass-spectrometry imaging, spatial transcriptomics, dissociative single-cell or single-nucleus profiles, cell-type identity, and tissue anatomy as complementary but non-equivalent evidence. The central argument is that spatial concordance should strengthen, but not close, biosynthetic inference, whereas spatial discordance can be diagnostically useful when competing explanations such as transport, temporal mismatch, turnover, or sequestration remain plausible. The model therefore separates evidence for molecular identity, cellular expression, anatomical position, chemical localization, biological movement, and orthogonal functional validation rather than collapsing them into a single confidence score. Medicinal-plant examples involving monoterpene indole alkaloids, taxoids, ginsenosides, artemisinin-related metabolism, and securinine-associated chemistry illustrate how cell-resolved and spatial evidence can refine pathway hypotheses. The framework remains bounded by analytical resolution, metabolite-identification confidence, cross-modal registration, transcript–metabolite timing differences, transport, tissue heterogeneity, and species-specific anatomy. Its intended role is hypothesis refinement and validation design, not causal proof from spatial co-occurrence alone.

Keywords: Spatial metabolomics, Medicinal plants, Biosynthetic pathways, Mass-spectrometry imaging, Spatial transcriptomics, Cell identity

How to Cite This Article: Ferraro L, Ricci M, Moretti G, Greco P. Spatial Evidence Changes Biosynthetic Inference: Integrating Mass-Spectrometry Imaging, Spatial Transcriptomics, Cell-Type Identity, and Tissue Anatomy in Medicinal-Plant Pathway Reconstruction. Spec J Pharmacogn Phytochem Biotechnol. 2026;6(1):22-30. https://doi.org/10.51847/aY0Vz57uGP

Introduction

Medicinal-plant pathway reconstruction has entered a phase in which the principal uncertainty is increasingly not only *which* genes encode relevant reactions but also *where* those reactions are organized within multicellular tissues. Single-cell and spatial technologies now provide routes for resolving specialized metabolism at cellular and anatomical scales that bulk measurements cannot directly preserve [1]. This shift is especially consequential for medicinal plants because their pharmacologically important natural products often arise from multistep pathways distributed among distinct cell states, tissues, or organs, making spatial multi-omics a potentially informative complement to biochemical and genomic reconstruction [2]. Spatial evidence, however, should not

be treated as an intrinsically superior form of proof. Its value depends on what is being measured, at what scale, and whether localization can be distinguished from synthesis, movement, or persistence.

The need for this distinction follows from the underlying biology. Specialized-metabolite pathways are embedded in layered transcriptional, regulatory, enzymatic, developmental, and environmental control [3]. At the same time, pathway inference remains dependent on the quality of medicinal-plant genome assemblies and annotations, which can remain incomplete or uncertain even when increasingly sophisticated sequencing resources are available [4]. Spatial measurements therefore do not replace sequence accuracy, biochemical identification, or functional assays. They add an additional evidential dimension: the relationship between molecular observations and native tissue organization.

This article develops a proposed spatial evidence-integration model for medicinal-plant pathway reconstruction. The model distinguishes five questions that are often conflated: whether a candidate reaction is chemically plausible; whether its associated genes are expressed in particular cells; whether those cells occupy relevant anatomical positions; whether pathway intermediates or products are detected in the same or different regions; and whether apparent mismatches can be explained by transport, turnover, storage, developmental timing, or measurement limitations. The aim is not to convert spatial concordance into causal certainty. Rather, spatial evidence is treated as a constraint on biosynthetic hypotheses: concordance can narrow plausible pathway organization, discordance can expose alternative explanations, and both require orthogonal validation before mechanistic claims are strengthened.

Biosynthetic inference without spatial context

Genomic and biochemical approaches can reconstruct substantial portions of natural-product pathways without explicitly resolving where the pathway operates in intact tissue. The Taxus genome, for example, has enabled extensive inference concerning paclitaxel biosynthetic genes and pathway organization [5]. Similarly, chromosome-level analysis of *Ophiorrhiza pumila* provided genomic and molecular evidence relevant to the evolution and organization of camptothecin biosynthesis [6]. Such studies show that pathway membership, gene-family evolution, and candidate enzymatic relationships can be investigated effectively even when cellular topology is incomplete.

Functional reconstruction can further strengthen reaction-level inference. Recent discovery of additional Taxol-pathway genes enabled reconstruction extending to baccatin III, demonstrating that enzyme discovery and pathway engineering can resolve missing chemical steps with substantial functional support [7]. In *Catharanthus roseus*, biochemical characterization of a BAHD acyltransferase established a specific 19-O-acetylation step within a tabersonine-derived monoterpene indole alkaloid branch [8]. These forms of evidence are central because spatial localization cannot compensate for an incorrectly assigned reaction.

Yet reaction identity and pathway topology answer different questions. An enzyme can be biochemically validated without establishing which native cell performs the reaction, and a heterologously reconstructed sequence of reactions need not reproduce the intercellular organization of the source plant. Likewise, genomic proximity or coexpression can support candidate relationships without demonstrating that substrate and enzyme meet in the same native compartment. Spatial evidence therefore changes biosynthetic inference by adding constraints on *where* compatible molecular events can occur and by revealing when apparently coherent pathway steps may instead be separated across cell types, tissues, or transport routes. The proposed model retains non-spatial evidence as the chemical and functional foundation, then asks whether native spatial organization supports, complicates, or redirects that reconstruction.

Different forms of spatial molecular evidence

Mass-spectrometry imaging (MSI) provides direct information about the distribution of detectable ions across tissue sections and has become an important component of plant spatial metabolomics [9]. Its principal contribution to pathway reconstruction is chemical geography: a candidate intermediate, product, or related metabolite can be associated with particular anatomical regions without physically homogenizing those regions before analysis. In-situ imaging approaches therefore preserve information that bulk extraction necessarily averages, but they remain snapshots whose interpretation depends on chemical assignment, ionization behavior, spatial resolution, and tissue preparation [10]. Reviews focused specifically on natural-product biosynthesis emphasize that MSI can help formulate or constrain pathway hypotheses, but localization alone does not identify the enzyme or demonstrate local flux [11].

Spatial transcriptomics contributes a different observable. Rather than measuring molecular accumulation, it maps RNA abundance within tissue coordinates. Cross-modal integration consequently requires registration, normalization, domain definition, and explicit handling of unequal spatial scales rather than assuming that transcript and metabolite maps are naturally commensurate [12]. Targeted approaches such as PHYTOMap demonstrate that gene-expression patterns can be resolved while retaining three-dimensional plant-tissue context [13]. More expansive plant atlases show that transcriptomic and other molecular layers can also be embedded within spatially resolved developmental frameworks [14]. These developments establish that anatomical position can become a measured variable rather than an inferred property of dissociated cells.

The important analytical point is therefore one of non-equivalence. MSI is closest to spatial chemical accumulation; spatial transcriptomics localizes RNA; single-cell profiles sharpen cell identity but may lack native coordinates; and anatomy supplies structural constraints within which all three must be interpreted. MSI and related in-situ approaches can localize plant metabolites and support biosynthetic hypothesis generation, but their interpretation remains constrained by chemical identification, sensitivity, resolution, and the distinction between observed localization and pathway flux [9-11]. Spatial integration is strongest when these differences are preserved rather than collapsed.

Cell-type and tissue-level concordance

Cell-resolved studies of medicinal plants demonstrate why anatomical context can materially alter pathway interpretation. In *Catharanthus roseus*, single-cell multi-omics resolved cell-type partitioning of monoterpene indole alkaloid metabolism, showing that specialized-metabolic information can be distributed across distinct cellular populations [15]. Subsequent cell-type-aware regulatory analysis further showed that regulatory landscapes can sharpen nomination and functional evaluation of pathway regulators [16]. Cell identity, however, should not be equated with a single biosynthetic function. Catharanthus idioblasts have been associated with alkaloid metabolism as well as broader stress-related functions [17]. More generally, single-cell analysis has shown that specialized-metabolic pathways can be organized across multiple cell types rather than confined to a single biosynthetic cell class [18].

Developmental state adds another layer. Single-nucleus analysis of *Artemisia annua* glandular trichomes resolved cellular differentiation relevant to artemisinin biosynthesis, illustrating that pathway-associated transcription can vary within a seemingly coherent anatomical structure [19]. Accordingly, concordance should be assessed not only between “gene” and “metabolite” but among gene expression, cell state, tissue position, and chemical distribution.

The strongest medicinal-plant examples combine molecular modalities. In Taxus leaves, MSI and single-cell transcriptional profiling revealed tissue-specific relationships between bioactive-metabolite distributions and cellular transcriptional programs [20]. Integrated analysis of Taxus stems likewise supported hypotheses involving both taxoid biosynthesis and movement between tissues [21]. In *Panax ginseng*, joint MSI and single-cell sequencing mapped multicellular compartmentation associated with ginsenoside metabolism [22], while integrated single-cell transcriptomics and spatial metabolomics in Panax root tips related cellular differentiation to spatially patterned ginsenoside biosynthesis [23]. Chemically guided single-cell analysis of securinine-associated metabolism further illustrates an important escalation principle: cell-resolved nomination becomes mechanistically stronger when followed by orthogonal chemical and functional validation [24].

Across these studies, concordance is best understood as evidence strengthening, not proof of local synthesis. Medicinal-plant examples show that combined cell-resolved transcriptional and spatial or cell-associated chemical evidence can narrow plausible relationships between pathway activity and tissue organization [15, 20, 21]. **Table 1** classifies the resulting evidence forms by what they observe, what inference they strengthen, and what uncertainty they leave unresolved.

Table 1. Spatial evidence classes and the biosynthetic questions they can and cannot resolve

Evidence class	Primary observable	Strongest supported inference	Principal unresolved question
MSI chemical localization	Tissue distribution of detected ions	Where candidate metabolites accumulate	Whether accumulation marks local synthesis, import, or storage
Spatial transcript localization	RNA abundance at tissue coordinates	Where pathway-associated transcription occurs	Whether transcript abundance yields enzyme activity or product formation

Dissociative single-cell profiling	Cell-resolved expression and identity	Which cell types express candidate pathway programs	Native position and intercellular movement
Tissue anatomy and cell identity	Structural location and cellular context	Whether molecular observations occupy biologically compatible compartments	Whether anatomical proximity permits actual substrate transfer
Cross-modal concordance	Agreement among chemical, transcript, cell, and tissue evidence	Narrower set of plausible pathway organizations	Causality, flux direction, timing, and transporter involvement
Orthogonal functional evidence	Reaction, perturbation, transport, or isotope response	Whether a nominated mechanism performs the proposed function	Generalization beyond the tested pathway and biological context

The classification also reveals a central asymmetry: concordant evidence can reduce the number of plausible pathway arrangements, whereas discordance may increase the number of biologically credible explanations.

Separating biosynthesis from transport and storage

Spatial localization becomes most difficult to interpret when the site at which a compound is detected is treated as synonymous with the site at which it was synthesized. Plant specialized metabolites and their intermediates can move through membrane transport, vascular transport, intracellular trafficking, or source–sink relationships, making transport biology integral to pathway reconstruction rather than an afterthought [25]. Consequently, a spatially enriched metabolite can represent local formation, import from another compartment, selective retention, or storage after synthesis elsewhere. The same problem applies to pathway intermediates: their movement can connect enzymatic steps that appear spatially separated.

Direct evidence from Taxus illustrates why this distinction matters. A Taxus NPF transporter has been shown to participate in uptake of 10-deacetylbaccatin III, with functional consequences for taxane biosynthesis [26]. Such evidence demonstrates that at least some natural-product pathways can depend on biologically meaningful intermediate movement. It does not imply that every transcript–metabolite mismatch reflects transport, but it makes transport an experimentally credible competing explanation when pathway components occupy different spatial domains.

This distinction changes how spatial concordance should be read. Co-localization of enzyme-associated transcripts and a pathway metabolite is compatible with local biosynthesis, whereas separation between them need not falsify the pathway assignment. Conversely, metabolite enrichment in a tissue with little detectable pathway transcription should not automatically be taken as evidence for an unrecognized local enzyme. Integrated Taxus evidence and broader transporter biology show that transport can separate sites of pathway-gene expression from sites of metabolite accumulation [21, 25, 26].

The proposed framework therefore treats biosynthesis, transport, and storage as competing spatial interpretations rather than sequential assumptions. Evidence for one interpretation should reduce, but not erase, the plausibility of the others. Local synthesis becomes stronger when reaction competence, cell-specific expression, anatomical compatibility, and local chemical detection converge. Transport becomes stronger when candidate source and sink compartments are biologically connected and transporter function is independently demonstrated. Storage or sequestration remains plausible when chemical accumulation persists without contemporaneous local biosynthetic evidence. These alternatives define the inferential problem that spatial integration must resolve.

A spatial evidence-integration model

Dissociative single-cell transcriptomics provides high-resolution cell identities but removes cells from their native coordinates, meaning that anatomical position must subsequently be reconstructed or supplied by another modality [27]. Spatial methods address that loss of position, yet they introduce their own limitations involving capture efficiency, resolution, registration, tissue accessibility, and computational integration [28]. Large plant spatial atlases demonstrate that cell identities can be anchored within anatomical and developmental contexts [29], while comparative technical analyses emphasize that single-cell and spatial platforms retain different strengths, biases, and scales [30]. The foundational rationale for plant spatial transcriptomics is therefore not that one modality supersedes another, but that positional information represents an independent biological dimension that dissociation alone cannot preserve [31].

On this basis, this article proposes a Spatial Evidence-Integration Model with four analytically separate evidential layers. The first is reaction evidence, comprising genomic nomination, biochemical assignment, and functional reconstruction. The second is cellular evidence, comprising cell type, cell state, and pathway-associated transcription. The third is anatomical evidence, specifying where those cells or domains lie within intact tissue. The fourth is chemical evidence, specifying where candidate intermediates and products are detected. None is treated as a surrogate for another.

Integration occurs through two interpretive operations. Concordance analysis asks whether independently measured layers occupy biologically compatible compartments. Discordance analysis asks which competing processes could explain their separation. The model does not convert these operations into a universal score because the evidential meaning of agreement depends on platform resolution, molecule identity, pathway chemistry, developmental state, and the possibility of transport. Instead, it produces a structured set of pathway hypotheses whose relative plausibility can be altered by new observations.

A second distinction separates inference from validation. Spatial co-occurrence may support a pathway arrangement, but stronger mechanistic attribution requires orthogonal evidence such as enzymatic conversion, genetic perturbation, transporter assays, isotope tracing, or repeated spatial measurements under informative conditions. The model is therefore iterative: validation can strengthen, weaken, or redirect an initially spatially plausible reconstruction.

Table 2 summarizes the resulting interpretation states and the evidence needed to distinguish them.

Table 2. Proposed interpretation states for integrating spatial transcript, cell, anatomical, and metabolite evidence

Observed evidence pattern	Plausible interpretation	Key unresolved alternative	Most informative validation direction
Transcript and metabolite overlap in compatible cells	Local biosynthesis is strengthened	Imported metabolite with local pathway expression	Enzyme or perturbation evidence
Transcript-rich, metabolite-poor region	Biosynthetic source with export or rapid turnover	Low product formation or analytical under-detection	Transport, time-course, or chemical confirmation
Metabolite-rich, transcript-poor region	Import, storage, or prior synthesis	Missed/low-abundance transcription	Transport and repeated spatial profiling
Adjacent but non-overlapping pathway steps	Intercellular pathway organization	Registration or resolution mismatch	Higher-resolution mapping and intermediate tracing
Strong cell identity but uncertain coordinates	Cell-type pathway nomination	Incorrect anatomical assignment	Spatial anchoring with tissue markers
Spatial concordance plus orthogonal function	Mechanistic interpretation substantially strengthened	Context-specific alternative remains possible	Replication across tissue/developmental contexts

Figure 1 places these states within the full proposed evidence architecture, including the point at which spatial

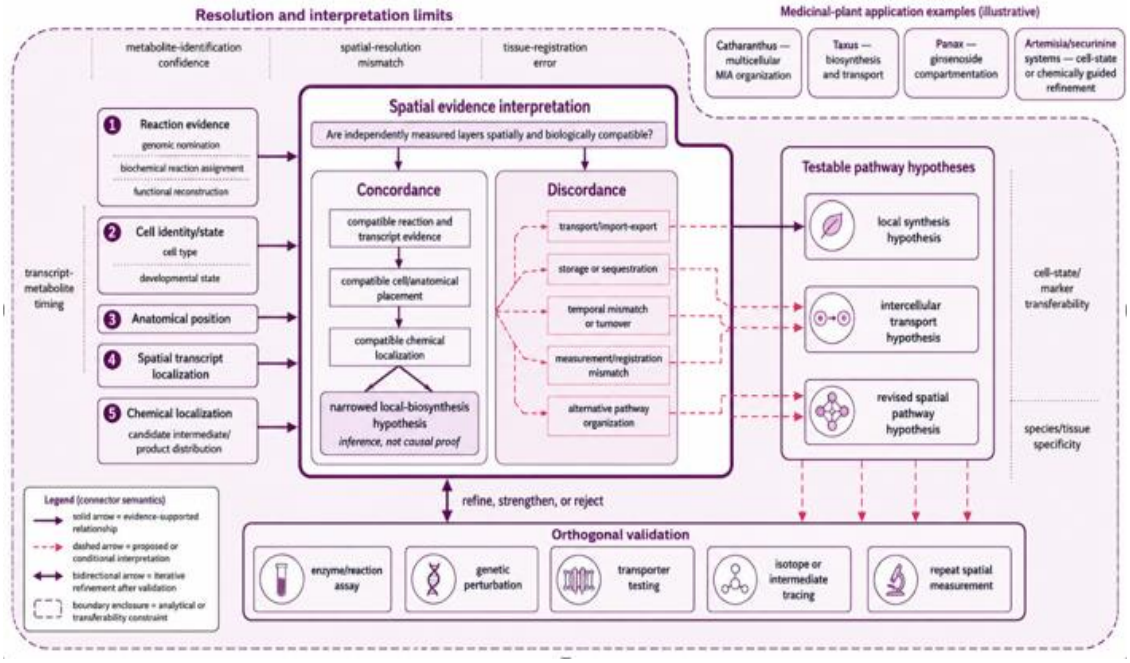

Figure 1. From spatial concordance and discordance to testable medicinal-plant pathway hypotheses

Using spatial discordance to refine pathway hypotheses

Spatial discordance is commonly treated as a nuisance produced by resolution mismatch or technical noise, but in pathway reconstruction it can also contain mechanistic information. A transcript-rich region with little corresponding metabolite signal may indicate export, rapid turnover, substrate limitation, developmental timing, or inadequate chemical sensitivity. A metabolite-rich region with weak contemporaneous pathway transcription may instead reflect import, storage, molecular stability, earlier expression, or incomplete transcript detection. The spatial model therefore treats discordance as a hypothesis-generating state, provided technical explanations remain explicitly represented.

The key analytical move is to ask which additional observation would discriminate among alternatives. In Taxus, combined cell-resolved and chemical evidence creates a context in which transport can be considered alongside local biosynthesis [21]. Transporter biology supplies a mechanistically plausible route for such separation [25], and direct functional characterization can move a transport interpretation from possibility toward pathway-specific evidence [26]. Discordance should consequently trigger targeted experiments rather than immediate pathway rejection.

The same logic applies when adjacent cell types express different sections of a pathway. Such a pattern may indicate genuine intercellular biosynthetic organization, but it can also result from misregistration, unequal detection sensitivity, or transient expression states. Chemically guided single-cell work demonstrates the value of following cell-based nomination with independent chemical or functional evidence [24]. The proposed model therefore assigns greater interpretive weight to discordance that survives orthogonal measurement than to discordance observed in only one platform.

Importantly, no fixed hierarchy of discordance patterns is proposed. A transcript–metabolite mismatch in a secretory structure may have different implications from the same mismatch in vascular tissue or a storage-rich organ. The purpose of discordance analysis is not to classify every mismatch automatically, but to make alternative explanations explicit and to design experiments capable of distinguishing among them.

Applications to medicinal-plant pathway reconstruction

The utility of the proposed model is clearest when applied comparatively rather than as a universal template. In *Catharanthus roseus*, cell-resolved evidence supports a multicellular organization of monoterpene indole alkaloid metabolism [15]. Cell-type-aware regulatory analysis adds another layer by identifying regulatory programs associated with pathway function [16], while idioblast biology cautions against assigning a cell type a single metabolic identity [17]. Single-cell pathway mapping further supports the broader principle that specialized

metabolism can span several cellular compartments [18]. Together, these observations favor reconstruction strategies that distinguish pathway step, regulatory state, and cell identity.

Taxus provides a different problem. MSI and single-cell transcriptional profiling reveal tissue-specific taxoid-related patterns in leaves [20], while stem studies explicitly connect spatial metabolic information with biosynthesis and transport hypotheses [21]. Functional evidence that an NPF transporter can take up a taxane intermediate adds a mechanistic basis for considering movement between compartments [26]. Thus, a Taxus reconstruction should not demand strict spatial coincidence between every pathway transcript and every detectable taxoid intermediate.

Panax illustrates the importance of tissue differentiation. Integrated MSI and single-cell evidence supports multicellular compartmentation of ginsenoside-associated metabolism [22], while root-tip analysis relates cellular differentiation to spatial metabolite patterns [23]. Artemisia further shows how developmental states within glandular trichomes can be relevant to specialized-metabolic interpretation [19]. These examples indicate that the spatial unit of inference may be a cell type, developmental state, anatomical domain, or interface between compartments rather than a single universally appropriate spatial scale.

Finally, chemically guided single-cell analysis of securinine-associated biosynthesis demonstrates the value of moving beyond co-localization toward orthogonal chemical and functional testing [24]. Across these systems, the model is therefore best used to organize evidence and competing explanations before intervention, not as an automated pathway-assignment rule.

Resolution limits and validation

Spatial evidence is only as informative as the measurements from which it is constructed. Native-tissue MSI can reveal distributions that bulk extraction would obscure, but chemical localization remains dependent on analytical identification, ion formation, sampling characteristics, and instrument-specific performance [32]. A spatial signal should therefore be interpreted as evidence that a compatible analyte-associated feature occurs at a location, not automatically as proof of molecular structure, reaction rate, or biosynthetic origin.

Cross-modal integration adds a second limitation: spatial units are rarely identical. MSI pixels, spatial-transcriptomic capture regions, in-situ transcript signals, and reconstructed single-cell identities can differ substantially in scale and information content [9]. Computational integration must therefore address registration and representation explicitly [12]. Tissue-preserving approaches such as PHYTOMap provide one solution for targeted expression localization [13], whereas broader multi-omic atlases demonstrate what becomes possible when cell identity and tissue coordinates are jointly modeled [14]. Even so, platform-specific uncertainty remains part of the inference rather than disappearing after integration [28].

Validation should consequently be matched to the uncertainty being tested. If chemical identity is uncertain, orthogonal chemical confirmation is required. If a candidate enzyme is uncertain, reaction assays or perturbation are more informative than further co-localization. If transport is proposed, transporter testing becomes central; direct Taxus transport evidence illustrates this distinction [26]. If a cell-guided candidate is proposed, chemically or functionally orthogonal follow-up can strengthen the assignment [24]. Dissociative single-cell findings may additionally require independent anatomical anchoring because native position is not retained during cell isolation [27].

The model should therefore remain falsifiable. A spatially coherent hypothesis can be weakened by failed enzyme activity, absence of expected intermediates, incompatible transporter evidence, or replication at higher resolution. Conversely, an initially discordant model can become stronger if transport, timing, or intercellular transfer is independently demonstrated. Spatial evidence changes biosynthetic inference most productively when it changes which experiment should be performed next.

Conclusion

Spatial evidence adds a dimension to medicinal-plant pathway reconstruction that cannot be reduced to another omics layer. Genomic and biochemical evidence can establish candidate genes, reactions, and functional pathway segments, whereas spatial measurements ask whether those assignments are compatible with native cellular and tissue organization. The central contribution proposed here is an integration model that keeps reaction competence, cell identity, anatomical position, transcript localization, chemical localization, transport, and orthogonal validation analytically distinct before combining them.

This separation is important because spatial concordance and spatial discordance have different evidential consequences. Concordance can reduce the number of plausible pathway arrangements but does not prove local synthesis. Discordance can expose transport, storage, temporal separation, turnover, or measurement limitations rather than simply falsifying a pathway. In both cases, the strongest inference arises when spatial observations direct discriminating biochemical, genetic, transport, chemical, or tracing experiments.

The model is intentionally evidence-bounded. Spatial resolution, chemical-identification confidence, registration accuracy, developmental state, tissue anatomy, transcript–metabolite timing, and species-specific transport biology constrain interpretation. Accordingly, the framework is not a validated scoring system, a universal pathway predictor, or a substitute for reaction-level functional evidence. Its value lies in making spatial assumptions explicit and turning agreement or disagreement among molecular layers into testable pathway alternatives. For medicinal plants with highly compartmentalized specialized metabolism, this shift from pathway membership to spatially constrained pathway organization may provide a more disciplined basis for reconstructing where biosynthesis actually occurs and for determining what evidence is still required.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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