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Seeing Phytochemistry in Place: A Scoping Review of Mass-Spectrometry Imaging for Spatial Localization of Specialized Metabolites in Medicinal and Bioactive Plants

Wei Zhang[1]*, Chen Hui[2], Michael Tan[1]

[1] Department of Mass-Spectrometry Imaging and Spatial Phytochemistry, Faculty of Pharmacy, University of Melbourne, Melbourne, Australia.
[2] Department of Spatial Metabolite Localization, Faculty of Pharmacy, National University of Singapore, Singapore.

***E-mail** ✉ wei.zhang@unimelb.edu.au

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ABSTRACT

Conventional extraction-based phytochemical analysis can identify many plant constituents but removes the anatomical context needed to determine where specialized metabolites accumulate, co-occur, or change within medicinal and bioactive plants. Mass-spectrometry imaging (MSI) addresses this spatial gap, although ion images differ substantially in chemical-identification confidence, quantitative meaning, and biological interpretability. This scoping review mapped peer-reviewed Q1 literature published from 2017 through 2024 on MSI-based localization of specialized metabolites in medicinal or bioactive plants, together with a limited set of transferable analytical and reporting studies required to interpret those applications. A DOI-unique candidate set of 52 records was screened using predefined eligibility and evidence-fit criteria, yielding 37 included articles. Study characteristics were charted across plant material, spatial scale, metabolite class, imaging modality, orthogonal validation, biological question, uncertainty, and transferability. The evidence shows a methodologically diverse field dominated by problem-specific analytical choices rather than a single preferred platform. MALDI-, DESI-, SALDI-, ambient-, and surface-aware approaches addressed different constraints involving tissue geometry, low-mass background, volatility, ionization, isomerism, and quantitation. Applications ranged from tissue-specific biosynthesis and developmental localization to cultivation, processing, material differentiation, and quality characterization. Stronger biological claims consistently depended on evidence beyond spatial co-localization. MSI can make phytochemistry anatomically explicit, but localization, molecular identity, abundance, and biological explanation should be treated as distinct evidential claims. The literature supports expanding spatial phytochemistry in medicinal plants while prioritizing claim-matched validation, reproducible sampling, and explicit interpretation boundaries.

Keywords: Mass spectrometry imaging, Spatial metabolomics, Medicinal plants, Phytochemistry, Specialized metabolites, MALDI-MSI

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Introduction

Medicinal and bioactive plants are chemically heterogeneous at scales that bulk extraction necessarily collapses. A root, leaf, flower, fruit, vascular region, secretory structure, or processed herbal material may contain the same nominal metabolite classes in markedly different local contexts, yet homogenization converts those distributions into a single composite measurement. MSI has therefore become increasingly useful for plant phytochemistry because it couples mass-resolved chemical information to anatomical position without requiring a separate label for each target. Recent synthesis of medicinal and edible plant applications shows that the field now spans multiple organs, compound classes, and analytical platforms, but also that performance remains strongly analyte- and method-dependent [1]. The central value of MSI is thus not simply “more metabolites”; it is retention of spatial

context that can be lost when chemistry is separated from tissue architecture. This distinction is especially important for pharmacognostic materials in which harvesting, processing, organ selection, or developmental state can change the anatomical compartment represented in a bulk extract. Spatial analysis can therefore complement conventional profiling by asking not only which constituents are detected, but where chemically informative signals reside.

Natural-product MSI literature further shows that this spatial context can support compound discovery, localization, and multimodal interpretation when imaging is combined with orthogonal chemical or biological information [2]. Plant-specific reviews, however, make clear that MSI is not one uniform technology: ionization mode, sample preparation, matrix chemistry, surface accessibility, spatial resolution, and molecular coverage differ across platforms [3]. These differences matter acutely in medicinal plants because specialized metabolites include structurally diverse alkaloids, terpenoids, phenolics, glycosides, and other low-molecular-weight compounds whose detectability and fragmentation behavior can differ substantially. Consequently, a chemically plausible ion image cannot be interpreted independently of the analytical conditions that produced it.

The biological attraction of spatial phytochemistry is equally strong. In situ plant-metabolism imaging can reveal tissue specialization and metabolite organization that bulk measurements cannot resolve, but meaningful interpretation still depends on chemical-identification confidence and biological context [4]. A localized ion can be consistent with biosynthesis, storage, transport, degradation, secretion, or exposure of a particular tissue surface; the image alone does not distinguish among these possibilities. This scoping review therefore treats spatial localization as an evidential starting point rather than an automatic mechanistic endpoint. Its purpose is to map how MSI has been used to localize specialized metabolites in medicinal and bioactive plants, compare the analytical strategies used to obtain those maps, identify the biological questions addressed, and determine where identity, quantitation, mechanism, quality interpretation, and transferability remain insufficiently validated.

Review scope and questions

The review was designed around spatially resolved phytochemistry rather than all uses of MSI in herbal medicine. Updated reviews of herbal-medicine MSI demonstrate a broad application landscape that includes chemical characterization, processing, distribution studies, and other analytical purposes [5]. The present scope is narrower: the primary evidence of interest is imaging that preserves a plant-related spatial coordinate while addressing specialized metabolites in medicinal or bioactive materials. Direct medicinal-plant studies form the core evidence. Non-medicinal plant or general MSI papers are retained only when they establish an analytical constraint—such as plant-tissue preparation, small-molecule ionization, structural discrimination, or quantitative interpretation—that the medicinal-plant literature does not adequately establish by itself.

Three distinctions govern interpretation. First, spatial detection is separated from molecular identification: an *m/z* feature may be localized confidently even when structural assignment remains incomplete. Second, relative signal distribution is separated from absolute abundance, because ion suppression, matrix effects, extraction behavior, and calibration can alter intensity. Third, spatial association is separated from biological mechanism. Co-localization of a metabolite with a tissue type, transcript, developmental state, or processing condition may strengthen a biological hypothesis without directly establishing biosynthetic flux, transport direction, enzyme causality, pharmacological action, or therapeutic effect. These boundaries are applied throughout the review rather than added only as limitations at the end.

Accordingly, the review asks: Which medicinal or bioactive plant materials, tissues, and specialized-metabolite classes have been spatially investigated? Which MSI approaches and preparation strategies were used, and what analytical problems were they intended to solve? What biological questions—biosynthesis, development, cultivation, processing, differentiation, or quality characterization—were addressed? What forms of orthogonal validation were used for identity, abundance, or mechanism? Finally, where do the included studies leave unresolved uncertainty that should shape future spatial-phytochemistry designs? The review is descriptive and analytical rather than effectiveness-oriented; it maps evidence characteristics and interpretive boundaries without pooling outcomes or assigning universal performance rankings to platforms. It also treats absence of an imaged signal conservatively: failure to visualize a compound can reflect true absence, concentration below detection, ionization suppression, preparation loss, surface inaccessibility, or an unsuitable acquisition mode. Accordingly, non-detection is not treated as evidence that a metabolite is biologically absent from a tissue.

Materials and Methods

The review followed a scoping-review design and used PRISMA-ScR as the reporting framework for transparent identification, screening, eligibility, and inclusion [6]. Eligibility was restricted to peer-reviewed journal articles published from 2017 through 2024 with a verifiable DOI, Q1 status in a relevant journal category and year, and a direct role in at least one analytical, biological, methodological, or interpretive claim. Direct medicinal- or bioactive-plant MSI studies were prioritized. General plant or MSI-method papers were eligible only when needed to establish a transferable limitation or validation principle. Non-spatial metabolomics, non-journal sources, duplicate versions, papers outside the date window, and studies lacking a claim-level role were excluded.

Data charting separated plant material and organ, developmental or processing context, metabolite class, MSI modality, sample-preparation strategy, spatial level, biological question, orthogonal analytical or biological validation, uncertainty source, and transferability boundary. Missing information was treated as unreported rather than inferred. The verified DOI-unique candidate ledger contained 52 screened records; 15 were excluded after eligibility and evidence-fit assessment, leaving 37 included articles. The exclusion reasons resolved into evidence redundancy or a superseded analytical role, adjacent or insufficiently aligned scope, and failure of the Q1 eligibility constraint. Raw source-specific database yields were not reconstructed from unavailable search exports and therefore are not represented as invented identification counts. Screening decisions were made at the article level after DOI de-duplication. Review charting was descriptive: studies were organized by analytical problem, application domain, validation depth, and interpretation boundary rather than combined statistically. Because the review question concerns the range and nature of spatial-phytochemistry evidence, no pooled effect estimate or cross-platform performance score was calculated.

The verified selection sequence is summarized in **Figure 1.**

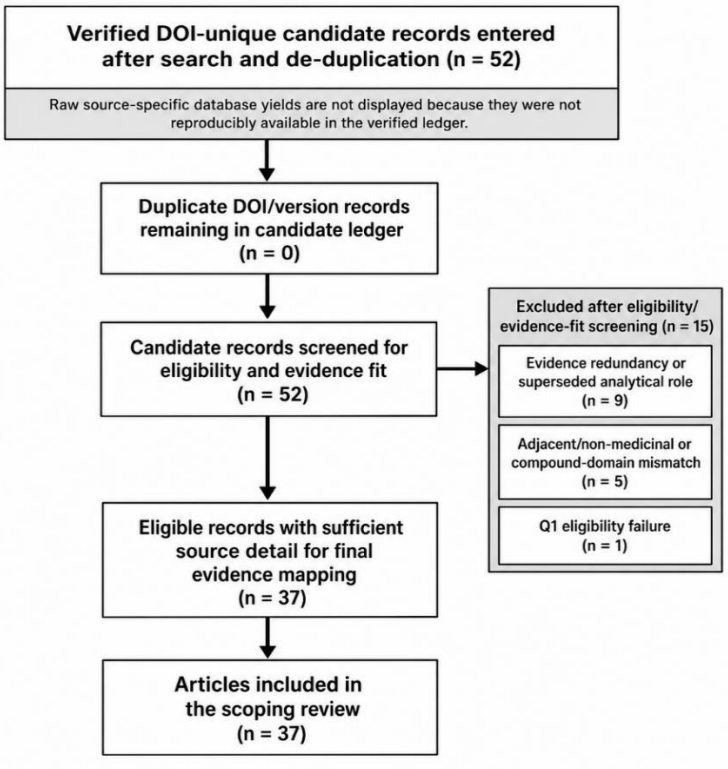

Figure 1. Verified evidence-selection flow from the DOI-unique candidate pool to the included scoping-review evidence

Table 1 organizes the evidence characteristics charted for each included article and the interpretive purpose of retaining them.

Table 1. Evidence characteristics charted to distinguish spatial description from stronger analytical or biological inference

Charted dimension	Recorded information	Analytical purpose	Interpretation boundary
Plant material	Species, medicinal/bioactive status, organ, tissue	Defines biological context	Material identity does not imply equivalent chemistry
Sampling context	Development, cultivation, processing, treatment	Captures major modifiers	Context association is not causation
Chemical focus	Metabolite class or targeted constituent	Links chemistry to method choice	Non-detection is not absence
MSI workflow	Ionization, matrix/derivatization, surface or section strategy	Explains analytical access	Platforms are not directly rankable
Spatial scale	Organ, tissue, surface, microregion	Defines localization resolution	Pixel localization is not cell identity
Identity support	Accurate mass, tandem MS, standards, orthogonal analysis	Grades assignment support	*m/z* agreement alone may remain ambiguous
Quantitative status	Relative imaging or calibrated measurement	Separates distribution from amount	Signal intensity is not inherently concentration
Biological validation	Omics, enzyme assays, functional or comparative evidence	Tests interpretation beyond localization	Association remains weaker than direct validation

Distribution and characteristics of the evidence

The included evidence was weighted toward recent work: 26 of the 37 included articles were published from 2022 through 2024. That concentration is consistent with the broader expansion of plant MSI described in recent field syntheses [1]. Older papers were retained selectively when they supplied a methodological or biological role that newer studies did not replace, including scoping-review reporting, native-surface imaging, isomer discrimination, quantitative MSI, and strongly validated tissue-specific biosynthetic localization. The resulting corpus should therefore be read as a deliberately bounded evidence map rather than as a census of all plant MSI research.

Study materials were heterogeneous. The corpus included roots and rhizomes, stems, leaves, petals and other floral tissues, fruits, aerial versus underground organs, seedlings, mature plants, and processed medicinal materials. Several studies compared developmental stages, cultivation conditions, related medicinal species, or processing times. This diversity is analytically important because plant anatomy is not a neutral container: sectionability, surface topology, tissue hardness, metabolite storage structures, and local matrix chemistry can all alter what is measurable. Consequently, the same nominal metabolite class can pose different analytical problems in a root cross-section, a leaf surface, or a processed herbal slice.

Methodologically, MALDI-based imaging was prominent, but the corpus also contained DESI, imprint-DESI, SALDI-type approaches, native-surface ambient ionization, and topography-aware surface imaging, matching the broader view that plant MSI is a toolbox rather than a single standardized workflow [3]. The biological purposes were similarly varied: descriptive localization, developmental change, putative pathway reconstruction, tissue-specific biosynthesis, cultivation-associated chemistry, processing transformation, species or material differentiation, and quality characterization. Orthogonal evidence ranged from none or limited chemical confirmation to LC-MS, tandem MS, metabolomics, single-cell transcriptomics, and biochemical enzyme validation. The principal heterogeneity is therefore not simply platform choice; it is the depth of evidence connecting a spatial signal to a chemical identity and then to a biological interpretation. This produced an uneven evidential landscape: some articles primarily demonstrated where selected ions occurred, whereas others coupled imaging to chromatographic confirmation, transcriptomics, metabolomics, or biochemical validation. The review therefore maps study characteristics along both an analytical axis—how the signal was generated and identified—and a biological axis—how far the study moved from localization toward explanation.

Analytical approaches used for spatial phytochemistry

Plant-specific sample geometry is a first-order analytical constraint. Irregular flowers, leaves, and roots can be difficult to planarize or section reproducibly, and a frozen-tissue planarization strategy improved access in tested specimens without establishing a universal preparation solution [7]. Low-molecular-weight compounds present a different problem: matrix background, ionization efficiency, and chemical interference can restrict MALDI-MSI

performance, motivating selective enhancement strategies [8]. On-tissue 4-APEBA derivatization expanded coverage for carbonyl-containing phytochemicals in tested plant tissues but remained functional-group dependent [9]. A related medicinal-plant example used derivatization to image volatile hemlock alkaloids whose localization would otherwise be difficult to preserve and detect [10]. Matrix engineering likewise improved low-background visualization of selected secondary metabolites in *Scutellaria baicalensis* roots, with orthogonal LC-MS supporting the analytical interpretation [11].

Other approaches alter how the tissue is accessed. Imprint DESI coupled with post-photoionization increased coverage and sensitivity for tested leaf metabolites, but transfer efficiency and possible delocalization remain relevant to spatial fidelity [12]. Laser-desorption low-temperature plasma imaging demonstrated direct analysis of native plant surfaces, reducing some sectioning and matrix requirements while retaining surface-access and ionization selectivity [13]. Functionalized TiO2 nanowire SALDI enabled imaging of selected vinca alkaloids in *Catharanthus roseus* petals [14], whereas 3D-surface MALDI preserved chemical information across irregular *Asclepias* leaf topography [15]. Structural and quantitative confidence require separate solutions: tandem MSI can discriminate isomeric plant metabolites that accurate mass alone cannot resolve [16], while quantitative MSI requires explicit calibration and control of matrix and ion-suppression effects rather than treating image intensity as concentration [17]. Finally, a high-coverage *Salvia miltiorrhiza* workflow illustrates the value of plant- and analyte-specific optimization while also showing why "higher coverage" should not be conflated with exhaustive metabolome capture [18].

Table 2 summarizes how these analytical strategies solve different problems while creating different interpretation boundaries.

Table 2. Analytical strategies used to extend spatial phytochemistry and the limitations that remain after method optimization

Analytical strategy	Primary problem addressed	Main analytical gain	Residual boundary
Tissue planarization/controlled sectioning	Irregular plant geometry	More reproducible spatial access	Tissue-specific transferability
Matrix optimization	Low-mass background, weak ionization	Improved signal for selected metabolites	Matrix–analyte bias
On-tissue derivatization	Volatility or poor ionization of target groups	Expanded targeted coverage	Reaction selectivity and response changes
Imprint DESI plus post-photoionization	Limited ambient-ionization sensitivity	Broader detected leaf chemistry	Transfer and delocalization
Native-surface ambient imaging	Sectioning or matrix dependence	Direct surface interrogation	Surface-biased chemical access
SALDI/nanostructured substrates	Matrix interference for selected analytes	Alternative desorption/ionization route	Substrate-specific selectivity
3D-surface MSI	Uneven native topography	Spatial mapping without flattening the surface	Instrument and registration demands
Tandem/quantitative MSI	Identity or abundance ambiguity	Stronger structural or concentration inference	Requires claim-specific validation

Biological questions addressed by imaging studies

The biological value of MSI becomes clearest when spatial localization is used to ask where specialized-metabolite pathways may operate. In *Salvia miltiorrhiza*, combining MSI with complementary metabolomics linked tissue distributions of flavonoids and phenolic acids to biosynthetic relationships, supporting tissue-level pathway hypotheses without directly measuring metabolic flux [19]. A later *Salvia* study integrated DESI-MSI with multi-omics across *S. miltiorrhiza* and *S. grandifolia*, connecting terpenoid distributions to biosynthetic candidates while leaving causal enzyme assignments dependent on functional validation [20]. That distinction is illustrated by *Platycodon grandiflorum*, where MSI-assisted spatial evidence was followed by genome mining

and biochemical characterization of a di-O-glycosyltransferase; the mechanistic strength came from the orthogonal enzyme evidence rather than from the image alone [21].

Cell-resolved integration adds another level of interpretation. In *Taxus mairei* stems, MSI combined with a single-cell transcriptome atlas associated taxoid distributions with cell populations implicated in biosynthesis and movement, but the data did not directly measure transport flux [22]. Related work in *Taxus* leaves linked spatial metabolite accumulation to cell-type-specific transcriptional programs and nominated regulatory relationships requiring further functional testing [23]. Strong tissue-level inference is also possible without single-cell sequencing when multiple evidence types converge: in *Hypericum perforatum* roots, high-resolution MSI integrated with molecular evidence supported the exodermis and endodermis as sites of xanthone biosynthesis [24]. By contrast, high-resolution MALDI and DESI imaging of *Rauvolfia tetraphylla* localized reserpine and proposed intermediates and thereby constrained a monoterpenoid-indole-alkaloid pathway, but spatial ordering did not itself prove enzymatic sequence [25].

MSI also addresses biological questions that are descriptive yet pharmacognostically important. *Gelsemium elegans* showed developmentally varying alkaloid distributions, although apparent redistribution cannot distinguish transport from local synthesis, degradation, or storage [26]. *Panax notoginseng* likewise exhibited changing spatial metabolite patterns across developmental contexts [27]. In *Scutellaria baicalensis*, comparison of aerial and underground organs revealed distinct spatial chemical organization [28]. DESI-MSI of Isatidis Radix demonstrated how tissue distributions can contribute to quality characterization without converting an imaged constituent into a validated quality marker [29]. Comparative spatial metabolomics differentiated ginseng from American ginseng chemically, not therapeutically [30]. Garden and forest-grown ginseng displayed different constituent patterns, but proposed inflammatory relevance remained separate from the imaging evidence [31]. Finally, steaming of Fuzi produced time-dependent alkaloid changes visualized by DESI-MSI and supported for selected compounds by HPLC, while clinical safety or efficacy remained outside what the chemical imaging established [32].

Current strengths and technical limitations

Across these studies, MSI's distinctive strength is multiplex chemical localization while preserving anatomical context. Its central weakness is equally important: a spatial signal, a molecular identity, a quantitative abundance, and a biological explanation are different evidential claims. This review therefore proposes a four-part interpretation boundary—localization, identity, abundance, and biological explanation—so that confidence in one layer is not automatically transferred to the next.

Analytical innovations broaden access to difficult chemistry, but they also add their own selectivity. Complementary preparation and ionization strategies broaden selected chemical coverage but do not create a chemically unbiased universal workflow [9, 12, 14]. Derivatization favors reactive functional groups; imprinting and surface ionization depend on transfer or surface accessibility; matrices and nanostructured substrates alter response. These constraints make absence of signal especially difficult to interpret.

Structural and quantitative limitations require different remedies. Isomeric metabolites may need tandem-MS evidence beyond accurate mass, whereas abundance claims may require internal standards, calibration, and explicit control of ion suppression. Plant anatomy adds another source of uncertainty because sectioning, flattening, and surface topography can alter access to chemically heterogeneous regions. Spatial resolution itself is also not equivalent to biological resolution: a pixel can contain several cellular compartments, while increasing nominal resolution can reduce available ion signal. Resolution must therefore be matched to the anatomical question and analytical sensitivity rather than maximized indiscriminately.

Spatial localization becomes mechanistically stronger when paired with omics or biochemical validation, but validation depth differs across studies [20-22]. The proposed hierarchy is therefore not "simple MSI versus advanced MSI"; it is whether the evidence supplied is appropriate for the claim being made.

The evidential distinctions emerging across the included studies are synthesized in **Figure 2**, which separates what an MSI observation directly establishes from progressively stronger claims that require additional analytical or biological validation.

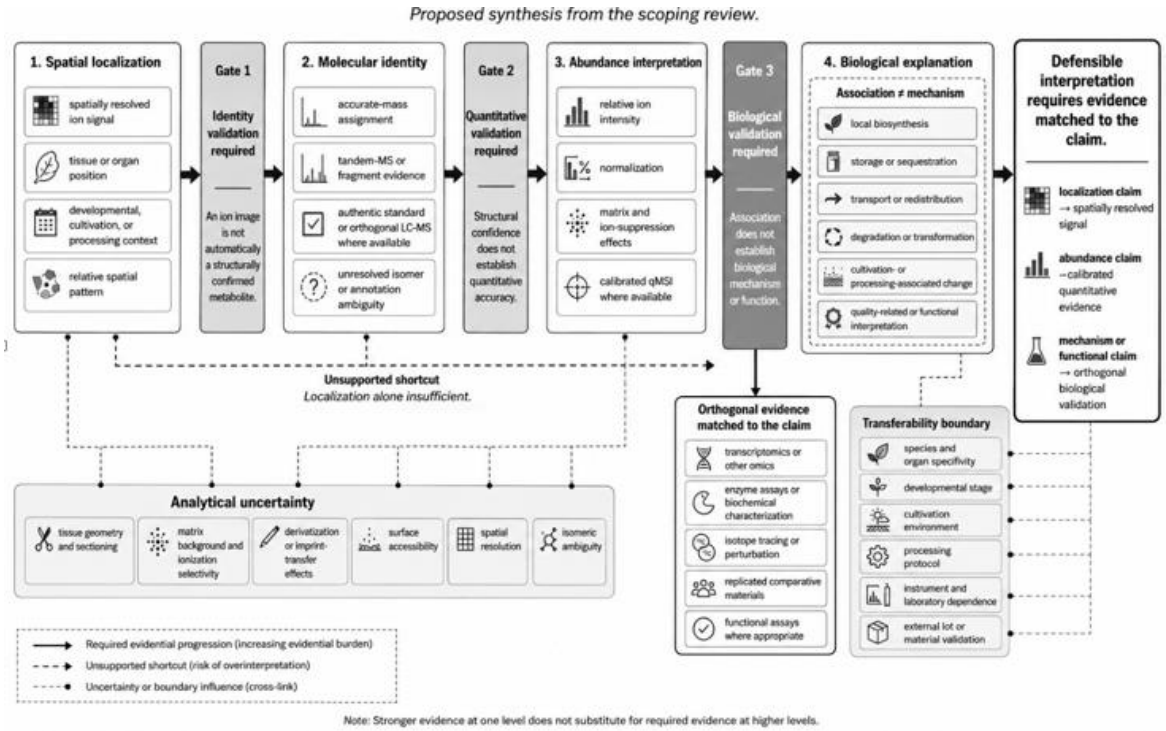

Figure 2. From spatial ion localization to defensible biological interpretation in medicinal-plant mass-spectrometry imaging

Underexplored medicinal-plant applications

Several application domains remain comparatively underdeveloped within the included evidence. Agronomic context is one. Continuous cropping of *Salvia miltiorrhiza* was associated with altered spatial metabolite distributions, showing that cultivation history can become visible at tissue level [33]. Yet soil chemistry, microbiota, climate, plant age, and site can covary with cropping history. Multi-site, multi-season replication is therefore a proposed priority before spatial signatures are treated as stable cultivation markers.

A second opportunity is representative organ and developmental sampling. Multi-organ imaging of *Clausena lansium* showed that alkaloids, coumarins, and other metabolite classes can occupy different plant regions [34]. Developmental MSI of wolfberry likewise demonstrated stage-dependent spatial chemical patterns [35]. These studies suggest that bulk comparisons based on one organ or one harvest point can miss meaningful heterogeneity. They do not, however, establish a universal "best" organ or developmental stage. Prospective stage- and organ-stratified designs would be more informative than treating those variables as incidental metadata.

A third opportunity is stronger linkage between spatial chemistry and independently measured function. In two Radix Puerariae species, AFADESI-MSI was combined with LC-MS and in vitro bioactivity analysis, providing a more defensible basis for interpreting chemical differences than imaging alone while still remaining preclinical [36]. This type of triangulation could be extended to authentication, cultivation comparisons, processing studies, and candidate quality markers. The relevant translational step is not to make MSI images stand in for pharmacology, but to use them to define where and which chemical differences deserve orthogonal testing.

Table 3 consolidates the principal application opportunities and the evidence boundaries that should accompany them.

Table 3. Evidence-supported opportunities and proposed validation priorities for medicinal-plant spatial phytochemistry

Application area	What MSI can contribute	Main unresolved issue	Proposed next-step evidence
Cultivation and chemotype studies	Tissue-level comparison of spatial chemical patterns	Site, season, age, and environmental confounding	Multi-site and multi-season replication

Organ and developmental sampling	Identification of heterogeneity missed by bulk extracts	Unequal or incidental sampling	Prospective organ- and stage-stratified designs
Processing studies	Localization of chemical transformation within material	Chemical change versus safety or efficacy	Orthogonal quantitation plus validated toxicological or pharmacological testing
Material differentiation	Spatial features distinguishing related medicinal materials	Batch and geographic transferability	External lot, provenance, and laboratory validation
Biosynthetic studies	Localization of metabolites and candidate pathway regions	Localization versus flux or enzyme causality	Isotope tracing, perturbation, or biochemical enzyme validation
Quality-marker research	Spatial context for candidate diagnostic constituents	Marker reproducibility and clinical relevance	Replicated analytical validation before quality or efficacy claims

Evidence gaps

The first major gap is comparability. Sample preparation, matrix chemistry, derivatization, ionization mode, spatial resolution, annotation criteria, and validation depth vary substantially across plant MSI studies. Some heterogeneity is scientifically appropriate because a volatile alkaloid, a surface glycoside, and an intracellular phenolic compound do not pose the same analytical problem. What is missing is sufficiently consistent reporting to distinguish genuine biological differences from method-specific visibility. A proposed minimum reporting set should therefore include tissue state and orientation, preparation and sectioning details, ionization conditions, spatial resolution, identity evidence, normalization or calibration strategy, and the status of orthogonal validation.

A second gap is scale. High-throughput MALDI-MSI of plant tissue microarrays demonstrated that larger and more reproducible screening designs are feasible, but homogenized tissue arrays answer a different spatial question from intact organs because native architecture is deliberately removed [37]. Scaling medicinal-plant MSI should therefore preserve a distinction between high-throughput chemical screening and native-tissue localization rather than treating one as a substitute for the other. Cross-laboratory reproducibility is also rarely a primary endpoint. Shared reference materials, harmonized metadata, and deliberate interlaboratory comparisons would help determine whether spatial signatures persist across differences in instrument, operator, matrix application, and acquisition settings.

Finally, validation should be matched to claim type. Identity, abundance, and mechanistic claims require different validation designs [16, 17, 21]. Authentic standards, tandem MS, or orthogonal separation can strengthen identity; calibrated qMSI can strengthen abundance; isotope tracing, perturbation, and enzyme assays are better suited to flux or causal pathway questions. For quality, cultivation, or authentication applications, independent lots, sites, and laboratories are additionally needed before transferability can be claimed.

Limitations of the review

This review is intentionally bounded. Restricting eligibility to peer-reviewed Q1 journal articles with verifiable DOIs from 2017 through 2024 improves consistency with the defined evidence standard but can exclude informative studies published in other journals, non-indexed sources, or work appearing after the search window. The evidence map should therefore not be interpreted as an exhaustive inventory of every plant MSI application.

The scoping design also limits the types of conclusion that can be drawn. The review maps study characteristics, analytical strategies, biological questions, validation practices, and evidence gaps; it does not estimate pooled effects, compare platform accuracy through meta-analysis, or formally grade certainty. Heterogeneity in plant species, tissues, analytes, preparation, ionization, spatial resolution, and study aims would in any case make simple cross-study numerical ranking misleading. Absence of a compound class or application from the included corpus may reflect the review's eligibility boundaries rather than a true absence from the wider literature.

The selection-flow evidence has a further reporting limitation. Candidate-level screening arithmetic was verified after DOI de-duplication, but reproducible raw source-specific database yields and cross-database duplicate counts were not available for reconstruction. Accordingly, the review reports only verified candidate-level counts and does not invent identification-stage numbers. Finally, many primary studies are exploratory and species-, organ-, or protocol-specific. Their spatial findings can motivate hypotheses and analytical priorities, but

generalization to other chemotypes, growing conditions, laboratories, processing regimes, pharmacological systems, or clinical outcomes requires additional validation.

Conclusion

Mass-spectrometry imaging has changed what can be asked of medicinal-plant chemistry by retaining the anatomical location of specialized-metabolite signals. Across the included evidence, that capability has supported questions about tissue specialization, developmental change, biosynthetic organization, cultivation, processing, material differentiation, and quality. The analytical diversity of the field is a strength because different tissues and compound classes require different solutions, but it also prevents a single platform or workflow from serving as a universal standard.

The central synthesis of this scoping review is that spatial phytochemistry should be interpreted through separate evidential layers. Localization identifies where a signal is observed; structural evidence determines what that signal most plausibly represents; quantitative design determines how abundance may be interpreted; and biological validation determines whether localization supports a mechanistic or functional explanation. Conflating these layers creates the largest risk of overinterpretation.

The practical implication is methodological discipline rather than restraint from using MSI. Spatial maps can remain highly informative when they are relative or only partially annotated, provided the evidential status is explicit. More reproducible sampling, transparent reporting, orthogonal chemical confirmation, calibrated abundance measurements, functional pathway tests, and external material replication would make spatial evidence more transferable. MSI is already a powerful means of seeing phytochemistry in place; its strongest future contribution will come from making clear not only where plant chemistry is observed, but how confidently that observation can be identified, quantified, explained, and applied.

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