



BIOCHEMICAL CHARACTERIZATION OF MEDICINAL PLANTS USING INTEGRATED OMICS TECHNOLOGIES

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Abstract: Alkaloids, terpenoids, phenolics, glycosides and other plant secondary metabolites form a very heterogeneous class; nonetheless, phytochemical analysis usually reveals only a small part of them. Omics technology unifies the disciplines of genomics, epigenomics, transcriptomics, proteomics, metabolomics, phenomics and microbiomics in order to establish a connection between genetic potential, enzymes, metabolites, and their functions. The present review provides an organized structure for the biochemical characterization of biological samples using the omics approach, especially with respect to experimental design, normalization, integration across platforms, pathway construction, and validation. Examples are given to highlight the importance of mathematical integration: Single cell analysis of *Catharanthus roseus* identified a 38 step biosynthesis pathway of monoterpenoid indole alkaloids in three leaf cell types; reconstruction of vinblastine synthesis pathway in yeast involved 30 enzymes and 56 genetic manipulations; while in *Artemisia*, 5.13 mg g⁻¹ dry weight of artemisinin corresponded to significant differences in gene-expression and trichome phenotypes. A workflow process that integrates authentic plant materials, sampling standardization, layer-based quality controls, scaling of features, network correlations, and functional validations that will lead to biomarker and bio-synthesis targets is suggested. The major limitations include partial genome, batch effects, lack of proper metabolites annotations, tissue heterogeneities, and inadequate causal validation. Innovative approaches in single cell sequencing, spatial sequencing, long read sequencing, and artificial intelligence can make medicinal plant characterization evolve from a mere descriptive approach to a biochemical predictive model.

Index Terms - medicinal plants, biochemical characterization, integrated omics, metabolomics, specialized metabolites, systems biology.

I. INTRODUCTION

Medicinal plants remain important sources of pharmaceutically useful components; however, the chemical makeup of these plants is variable and not static. This chemical makeup is determined by an interaction between genotypes, growth stages, tissue types, cellular type, soil, climate, microbiota, harvest time, and subsequent treatment (Vieira et al., 2024). The classical analysis methods, such as determination of total phenol concentration, chromatography, and the activity of certain enzymes, still hold relevance; but these methods alone cannot answer why a particular compound accumulates, which genes and enzymes are responsible for its synthesis, and what stimuli shift the metabolic flux to produce it (Keurentjes et al., 2006). The application of integrated omics can fill this void because it involves analyzing several molecular levels on a biological system and correlating them with a known biochemical phenotype. The goal is not necessarily to have an extensive amount of data but to create traceable correlations between the DNA sequence, transcript, protein abundance/activation, metabolites, and biology. A comprehensive multi-omics experiment always starts off with an authenticated plant sample together with a biochemical problem that

has been well defined, for example, why a certain chemotype produces more artemisinin, how the root-specific alkaloid pathway is compartmentalized, or what makes the real samples from the fake ones (Scossa et al., 2018). New research at single-cell and spatial scales shows that average numbers at organ level may hide pathways segregation in neighboring cells, while long-read sequencing and improved chromosomal assemblies expose duplicated genes and gene clusters for biosynthesis that were formerly broken into fragments.

This selective review covers representative advances, which have been mainly documented between 2018 and 2025. It elaborates on the role played by each layer of omics and discusses the quantitative equations of integration, workflow, as well as numerical data of relevant cases of medicinal plants. The stress is on biochemical profiling rather than mere sequencing.

II. REVIEW METHODOLOGY

Integrated omics approaches used for the biochemical characterizations of medicinal plants have been examined using a targeted narrative review approach. Peer-reviewed journal articles and reviews covering the fields of genomics, epigenomics, transcriptomics, proteomics, metabolomics, phenomics, microbiomics, spatial omics, and metabolic engineering have been included. The approach of a thematic review was used to examine the contribution of integrated omics tools to the biochemical investigation of medicinal plants. Research publications and review papers related to genomics, epigenomics, transcriptomics, proteomics, metabolomics, phenomics, microbiomics, spatial omics, and metabolic engineering were included.

Selection criteria were based on the use or application of two or more layers of data, molecular, biochemical, phenotypic, or computational, in medicinal-plant research. In particular, special attention was paid to works where some quantitative data associated with metabolites, genes, enzymes, genome, pathways, or experimentation were presented. Neither was any importance attached to work involving pure identification through taxonomy or sequencing of genome without biochemical elucidation. Literature for synthesis was categorized under the themes of omics layers, analysis integration, use of medicinal plants, quality assurance needs, present limitations, and future perspectives. This approach was intended to provide a concise and critical overview rather than an exhaustive systematic review.

III. OMICS LAYERS FOR BIOCHEMICAL CHARACTERIZATION

Different omics layers collectively connect genetic information, gene expression, enzyme activity, metabolite production, and observable plant traits. Their integration provides a comprehensive understanding of the biochemical pathways responsible for medicinally important compounds.

3.1 Genomics and Epigenomics

The genomics reveal the potential chemical properties of the biochemistry present in the medicinal plants. The better the genome assembly is, the easier it is to determine enzymes, transporters, transcription factors, tandem duplications, and gene clusters of the biosynthesis. Comparative genomics helps separate conserved pathways from unique lineages. For example, genome-wide screening for WRKY transcription factor family genes in *Aquilaria sinensis* revealed 70 WRKY genes, seven of which were found to be associated with the development of agarwood. Yet, one has to proceed with care when interpreting genomics: lack of a gene coding for a pathway component might be due to technical limitations rather than true absence (Xu et al., 2013). In the 2025 assessment, there were found a number of unvalidated genes for some published medicinal-plant genomes, and importantly, a telomere-to-telomere assembly of *Peucedanum praeruptorum* improved the N50 size from 11 Mb to 160 Mb and BUSCO score from 96.0% to 98.2%. In other words, it shows how assembly quality affects pathway prediction. Epigenomic studies include DNA methylation, histone modifications, and chromatin accessibility. They add a layer of regulation to explain why catalytically functional genes are expressed selectively in certain organs, developmental stages, and stress conditions (Lawrence et al., 2015).

3.2 Transcriptomics

Phytochemical screening provides an indication of broad types of secondary metabolites but lacks molecular specificity and quantification. RNA sequencing provides a direct method for assessing metabolic-pathway activity by measuring transcript abundance. The differential expression analysis allows identification of genes responsive to elicitor, pathogen, drought, light, or developmental cues. In contrast, the co-expression methods such as the weighted gene co-expression network analysis group genes that share similar expression profiles and relate the modules to target metabolites (Cortijo et al., 2020).

Association of candidate genes is made stronger where the transcript levels co-vary with a metabolite in several organs or treatments. However, transcript level does not correlate directly to enzyme activity since various other considerations like protein turnover can affect their association. The heterogeneity of the tissue is another limitation. In *Catharanthus roseus*, single cell RNA sequencing showed that there is a 38 step process of monoterpene indole alkaloids pathway, which is divided into three leaf cell types in a sequential order.

3.3 Proteomics and Enzyme-Level Characterization

Proteomics provides information on the actual presence of enzymes, transporters, and regulators within an organ or a sample. Techniques include the use of label-free LC-MS/MS, tandem mass tag labeling, and selected reaction monitoring for quantification. Post-Translational modifications such as phosphoproteomics are particularly important when the mechanism of regulation is dependent on fast signaling (Arsova et al., 2018). The major problem is that there are many plant-specific enzymes which have low abundance, are membrane-bound, or not represented in the database. Consequently, proteomic evidence is strongest when supported by a species-specific genome or transcriptome and validated by enzyme assays, heterologous expression or gene editing.

3.4 Metabolomics and Metabolic Imaging

Metabolomics is the omics domain closest to the medicinal phenotype. Un-targeted analysis by LC-MS, GC-MS, and NMR provides detailed chemical profiles, while targeted analysis involves quantification of known alkaloids, terpenoids, flavonoids, or saponins using authenticated standards (Allard et al., 2018). Mass measurement, isotope distribution, fragmentation, retention time, and spectral databases need to be combined because mass alone will hardly prove the identity of the analyte. Metabolomics with spatial resolution and imaging mass spectrometry keep tissue localization intact, and single-cell metabolomics allows linking of pathway intermediates to cell types. Single cell metabolomics along with single cell transcriptomics enabled the characterization of a reductase in *Catharanthus roseus* that is involved in the biosynthesis of anhydrovinblastine, thus establishing that the spatial coincidence between them turns correlation into an enzyme hypothesis (Singh et al., 2022).

3.5 Phenomics, Microbiomics and Bioactivity Data

Phenomics relates molecular profile data to measurable phenotypes such as glandular-trichome abundance, biomass, root systems, resistance to disease, and extract yield. Microbiomics is becoming increasingly important as endophytic and rhizospheric microbiota have the potential to alter precursors, induce defense responses, or even manipulate plant metabolites. Bioactivity studies, which include assays like antioxidant, antimicrobial, anti-inflammatory, cytotoxic, or enzyme inhibitory assays, need to be considered an extra quantitative dimension, not as a separate end point. This method enables the establishment of correlations between metabolites and bioactivities, but proving causal relationships still needs purified compounds and dose-response experiments (Covington et al., 2016).

IV. INTEGRATED ANALYTICAL FRAMEWORK

Integration needs to be envisioned as starting before data collection. The requirements for harmonization are biological replicates, organ type, developmental stage, harvest time, storage, order of extraction, and batch designation. The use of randomization and pooled quality control samples is used to avoid confounding. The layer filtering comes before the scaling and normalization process in order to prevent high abundance metabolites from having undue weight on low-level data. For a feature x_{ij} from sample i and omics layer j , z-score normalization is commonly expressed as:

$$z_{ij} = (x_{ij} - \mu_j) / \sigma_j \quad \square \square \square$$

where μ_j and σ_j are the mean and standard deviation of feature j . Differential changes can be summarized as log₂ fold change, $\log_2\text{FC} = \log_2(x_{\text{treatment}}/x_{\text{control}})$, so that +1 and -1 represent twofold increase and decrease, respectively. After scaling, a transparent weighted evidence score for candidate pathway element k can be written as:

$$S_k = \sum_m w_m z_{km}, \quad \sum_m w_m = 1 \quad \square \square \square$$

where z_{km} is the standardized evidence for candidate k in omics layer m and w_m is a prespecified or cross-validated weight. Such a score is not a substitute for biology; it is a reproducible way to prioritize

candidates supported simultaneously by gene expression, protein abundance, metabolite correlation and phenotype. Networks can be integrated through correlation, partial correlation, mutual information, and Bayesian measures. The safe approach would be to keep only those connections that are consistent and validated across separate batches of data with pathway knowledge, rather than choosing connections from a threshold.

When a stoichiometric metabolic model is available, steady-state flux analysis imposes mass balance:

$$S \cdot v = 0, \quad v_{\min} \leq v \leq v_{\max}, \quad \text{maximize } c^T v$$

The matrix of the stoichiometric relations in reactions is denoted by S , the vector of the reaction flows by v , and the objective to optimize by c (e.g., maximal output of a certain medicinal metabolite). The transcriptomic and proteomic data can provide constraints on the reaction bounds, and the measured levels of the metabolites can be used for verification. Multivariate approaches such as principal component analysis, partial least squares regression, canonical correlation, sparse generalized canonical correlation, multimomics factor analysis, and supervised machine learning can be effective tools, although their accuracy needs to be assessed via nested cross-validation and, if possible, via external data validation.

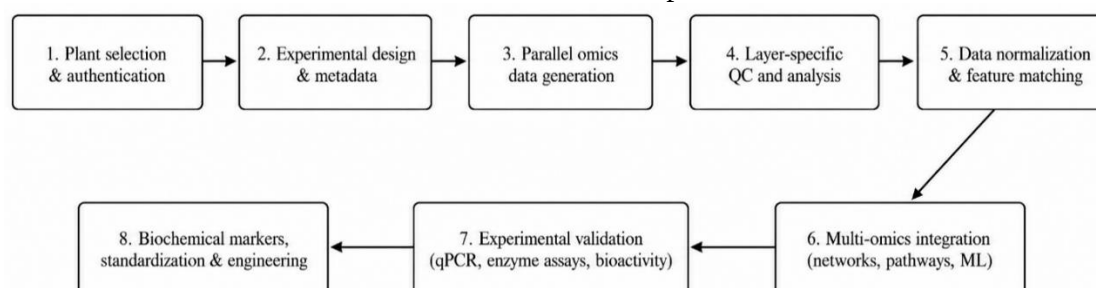


Fig. 1: Integrated workflow for biochemical characterization of medicinal plants using multi-omics

Figure 1 illustrates the sequential workflow for the biochemical characterization of medicinal plants, beginning with plant authentication and standardized experimental design, followed by parallel omics-data generation, quality control, normalization, and feature matching. The integrated datasets are subsequently analysed using network, pathway, and machine-learning approaches, validated experimentally, and translated into biochemical markers, quality-standardization tools, and metabolic-engineering targets.

V. INTEGRATED MULTI-OMICS WORKFLOW AND COMPUTATIONAL ANALYSIS

An integrated multi-omics workflow combines standardized sampling, layer-specific data generation, preprocessing, normalization, and computational integration to identify biologically meaningful relationships. Statistical modelling, network analysis, pathway mapping, and machine-learning methods are then used to prioritize candidate genes, enzymes, metabolites, and biomarkers for experimental validation.

Table 1: Quantitative examples showing the analytical value of integrated omics

System	Integrated evidence	Key numerical insight	Biochemical interpretation
Artemisia species / artemisinin	qPCR + metabolite assay + trichome phenotype	A. deserti: 5.13 mg g ⁻¹ DW artemisinin; Aa-CPS and Aa-GAS expression 77.81- and 75.29-fold above comparator. A. khorassanica: 4 trichomes mm ⁻² and no detected artemisinin.	Competing branch enzymes and glandular-trichome biology jointly redirect sesquiterpene flux.
Catharanthus roseus / MIAs	Chromosome assembly + Hi-C + single-	Eight chromosomes; 38-step pathway	Cellular compartmentation and transport are

	cell RNA-seq + single-cell metabolomics	distributed across three leaf cell types; reductase linked to anhydrovinblastine formation	essential components of pathway reconstruction.
Yeast reconstruction of <i>C. roseus</i> pathway	Plant omics-guided metabolic engineering	30 enzymatic steps, 56 genetic edits and 34 heterologous plant genes used to produce catharanthine and vindoline precursors.	Integrated pathway knowledge can transfer rare-plant chemistry into scalable microbial production.
<i>Aquilaria sinensis</i> / agarwood	Genome + RNA-seq + qPCR	70 WRKY genes identified; seven prioritized as likely regulators of agarwood biosynthesis.	Genome-wide families can be narrowed to experimentally tractable regulators.
<i>Peucedanum praeruptorum</i> genome quality	Chromosome-level versus T2T assembly	Contig N50 increased 11→160 Mb; BUSCO increased 96.0→98.2%.	Assembly completeness materially changes biosynthetic-gene-cluster discovery.

Table 1 presents representative medicinal-plant systems analysed through integrated omics approaches, highlighting the molecular evidence, quantitative findings, and corresponding biochemical interpretations. The comparison demonstrates how different combinations of genomic, transcriptomic, metabolomic, and phenotypic evidence support pathway identification and candidate prioritization.

VI. APPLICATIONS IN MEDICINAL-PLANT RESEARCH

Integrated omics supports medicinal-plant authentication, quality standardization, pathway discovery, and identification of bioactive compounds. It also facilitates metabolic engineering, conservation, and sustainable production of valuable phytochemicals.

6.1 Authentication and Quality Standardization

The genomic barcoding technique as well as single nucleotide polymorphisms are useful in species identification, while metabolomics is used for identifying substitutions and damage. The best quality control panel is one that combines stable and chemical markers together (Abraham & Kellogg, 2021). For example, a DNA marker will confirm the species but not ensure that the extracted organ has the expected quantity of active metabolites. On the other hand, the metabolite marker is likely to be influenced by storage and environmental conditions. Thus, multi-omics approaches support the application of a dual requirement for validity: genetic genuineness alongside biochemical efficacy. The machine learning classifiers must output sensitivity, specificity, balanced accuracy, and confidence intervals and not just accuracy, especially when faced with class imbalances.

6.2 Pathway Discovery and Metabolic Engineering

The most important aspect of integrated omics in terms of research is that of candidate reduction. The genome in question could potentially have hundreds of genes coding for cytochrome P450s, glycosyltransferases, and oxidoreductases; but only a few of those will be expressed at the same time as the metabolite being studied. Using co-expression, gene proximity, protein data, and metabolites correlation leads to an ordered list that will be used in further functional experiments (Orth & Palsson, 2010). *C. roseus* is a good example of full-scale pathway translation: omics technologies help in

determining cell organization and potential enzyme candidates, whereas existing pathway data enables engineering of 30-step yeast pathway to produce vinblastine precursors. Similar strategies can improve artemisinin, ginsenoside, tanshinone, taxane and alkaloid production through breeding, elicitation, tissue culture or heterologous biosynthesis.

6.3 Mechanism of Action and Network Pharmacology

Many extracts have several different substances acting on various targets. Combination of plant metabolomics analysis with cellular or organism response in terms of transcriptomics, proteomics, or metabolomics allows one to identify altered pathways. The network pharmacology approach links compounds, targets predicted by computation, and disease pathways; yet, computation-based correlations cannot be considered proof. The proof would need to include validation with respect to compounds, realistic exposure, target binding/activation/inhibition, pathway modulation, and dose-dependent effects. Integrated omics is most useful when it narrows mechanisms for validation rather than replacing pharmacology (Du et al., 2024; Pilarczyk et al., 2022).

6.4 Conservation and Sustainable Production

However, many high-value medicinal plant species have slow growth, are found in restricted habitats, or produce bioactive metabolites in low concentrations. Omics-assisted marker selection can determine productive chemotypes, while studies on transcript-metabolite interactions may help fine-tune harvest timing and elicitation protocols. Profiled endophyte and rhizosphere may reveal the presence of microbes capable of increasing biomass or secondary metabolite production (Aghdam & Brown, 2021). After complete characterization of the pathway, plant cell cultures, hairy-root cultures, and engineered microbes can be employed to reduce harvest pressure on natural populations. Sustainability claims should nevertheless include yield, energy, solvent use and life-cycle impacts; a higher laboratory titre is not automatically a lower-impact production process.

VII. LIMITATIONS AND QUALITY-ASSURANCE REQUIREMENTS

The major difficulty arises from the fact that the genetic data of many medicinal plants is not fully sequenced. The primary problem is that of incomplete and biased genetic reference data. The medicinal plants are diverse from an evolutionary perspective, they often have high heterozygosity or ploidy, and they have large genomes with many repeats. According to the 2025 genome quality assessment, experimentally verified pathway genes could not be found in genome assemblies and annotations. Second, it is difficult to combine the data from metabolomics of mature roots and from transcriptomics of young leaves due to possible sample mismatches. Third, artificial clusters can be formed due to batch effects, ion suppression, missing data points, and non-normalization issues. Fourth, one difficulty that exists is that many plant metabolites do not have standard reference substances or mass spectrometry data for identification. Fifth, correlation cannot prove causation because the correlated metabolites may have other factors in common.

The minimum standard for quality must include taxonomy confirmation and voucher archiving; a minimum of three biological replicates for exploratory studies, sample-size calculations based on power analysis for confirmatory studies; randomization of extraction and sequencing/mass spectrometry ordering; quality control pooling; missing values management; false discovery rate adjustments; archiving of raw sequencing and mass spectrometry data; recording of software version numbers and parameters used; and validation of high priority targets. For metabolomics, identification confidence should be stated explicitly, separating authentic-standard confirmation from tentative database annotation. For predictive models, feature selection must occur inside cross-validation to prevent information leakage.

VIII. EMERGING DIRECTIONS

Single-cell and spatial multi-omics will become more useful for unraveling the rare secretory cells that make up most of the specialized metabolic functions even though they are a tiny part of the organ. Long-read transcriptomics can aid in the discrimination of isoforms and improving gene annotation, whereas telomere to telomere assembly will be helpful in understanding duplicated pathway genes and gene cluster structures. Isotopomer-resolved metabolomics helps shift from correlational studies to flux analysis, and CRISPR perturbations allow testing causation. Artificial intelligence may help with spectral identification, cross-modal embedding, and prioritization of candidates, but explainability and uncertainty need to be considered since there is an uneven distribution of training data among different species and chemicals. A

viable short-term approach is the creation of digital twins for medicinal plants: computational models that combine genetics, environment, plant growth stage, and omics data to predict yields and product quality before harvesting.

Another important aspect to consider is standardization among laboratories. The use of common reference samples, standardized metadata, retention time standards, public spectral databases, and benchmark sets will improve transferability of models. In future review and experiments papers, distinction should be made between discovery, replication, and validation stages. While discovery can afford wide and non-targeted profiling, any claims that are made towards quality control and therapeutic applications must involve targeted measurements, external samples and validation of function.

IX. CONCLUSION

Integrated omics have revolutionized medicinal plant chemistry from mere compound enumeration to the elucidation of how compounds are actually produced. Genomics is used to determine biochemical potential; transcriptomics helps in determining pathway induction; proteomics provides insights into enzymes and regulation; metabolomics provides details on the medicinal phenotype; and spatial, phenomic, and microbiome profiling provide context. The most solid studies combine these diverse layers using standardized sampling, normalized analysis, reliable statistical analyses, and experimentation. Examples from *Artemisia*, *Catharanthus*, *Aquilaria*, and *Peucedanum* show how multi-omics techniques have the capacity to discover conflicting fluxes, cellular compartmentation, pathway length, regulatory families, and genome quality issues. Used rigorously, such an approach holds promise for improving areas like authentication, quality control, drug leads, metabolic engineering, and sustainable production. Without the help of samples and validation, the chances exist of obtaining an attractive but causation-free network. Therefore, the next stage of work must concentrate on obtaining high-quality literature citations, higher spatial resolution, isotope-derived flux information, and predictive models.

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XI. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this review article.

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