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Review

Biotechnological Interventions in Enhancing Secondary Metabolites from Medicinal Plants: A Review

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Abstract

Secondary metabolites isolated from medicinal plants are of immense therapeutic, nutraceutical, and market importance. Unfortunately, natural biosynthesis of these compounds is generally low, dependent on factors that include the environment and physiology. Over the past few years, biotechnological innovation has opened up new and sustainable approaches to meet the requirement for secondary metabolite production in vitro. This review identifies recent biotechnological interventions such as plant tissue culture, elicitation, metabolic engineering, and synthetic biology that have been successfully utilized to enhance the biosynthesis of bioactive phytochemicals. An emphasis is given to the mechanisms, benefits, and disadvantages of these strategies, with specific reference to prominent medicinal plant species. Future directions are to interface omics tools with AI-powered metabolic pathway modeling for targeted metabolite improvement.

Keywords: secondary metabolites; medicinal plants; tissue culture; elicitation; metabolic engineering; synthetic biology; hairy root; plant biotechnology

1. Introduction

Medicinal plants have been employed for thousands of years in conventional as well as contemporary medicine because they possess a rich treasure trove of bioactive secondary metabolites like alkaloids, flavonoids, terpenoids, and phenolics (Twaij and Hasan, 2022; Rauf et al., 2025). These metabolites are synthesized through complex biosynthetic pathways, often at low levels. Environmental stress, growth stage, and genetics play a huge role in their accumulation. For these reasons, an increasing interest exists in applying biotechnological means to overcome these challenges and provide sustainable production (Figure 1).

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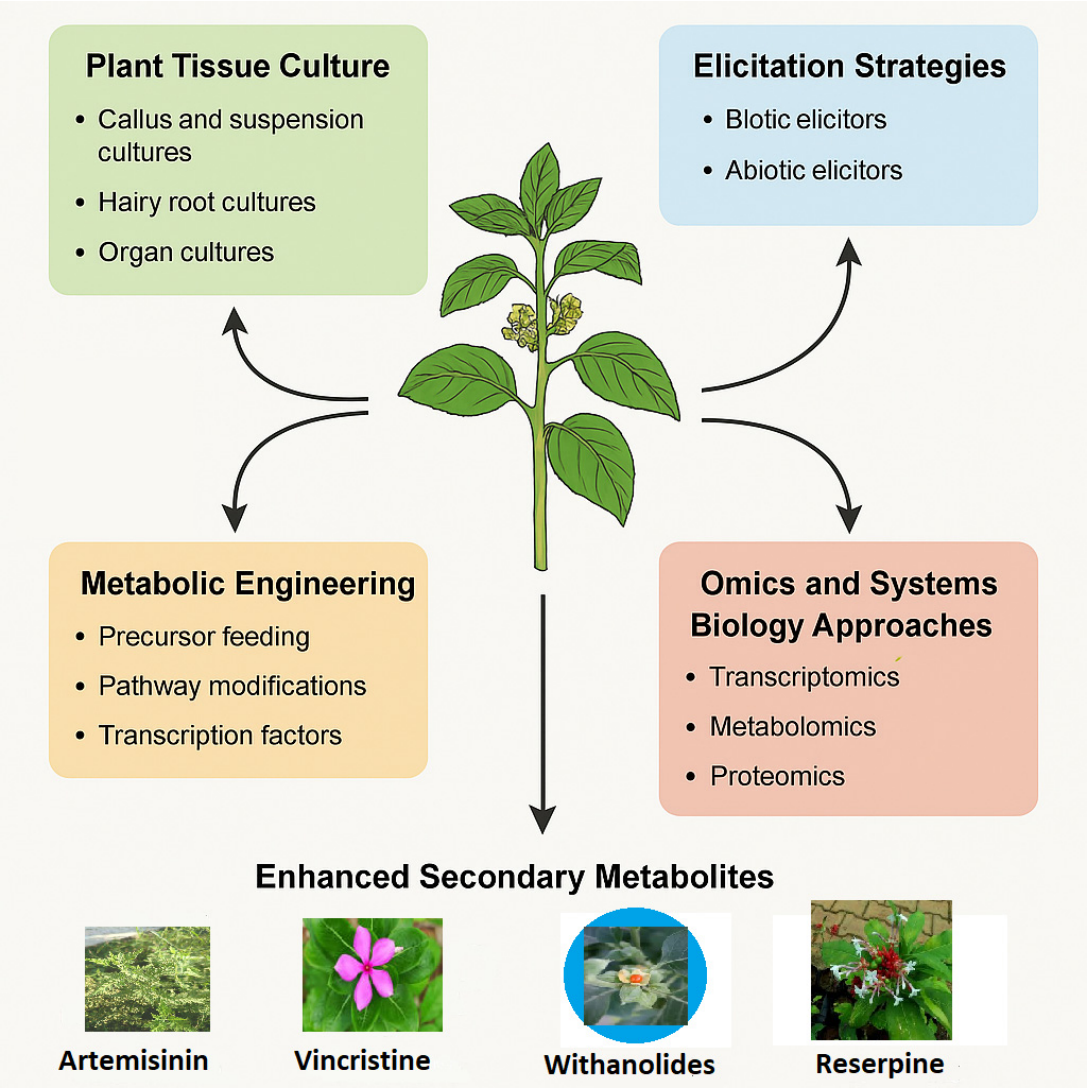

Figure 1. Biotechnological Strategies in Secondary Metabolite Enhancement.

1.1. Role of Plant Tissue Culture

Plant tissue culture (Karuppusamy, 2009; Fatima et al., 2023 is an exemplary technology that offers a platform in controlled conditions for secondary metabolite production (Nordine, 2025). Platforms most frequently used are callus and suspension cultures, shoot cultures, and hairy root cultures (Liu et al., 2025) (Figure 2).

- **Callus and Cell Suspension Cultures:** Sufficient for the production of uniform biomass. e.g., Taxus spp. cell cultures for the production of paclitaxel (Tomilova et al., 2023).
- **Hairy Root Cultures:** They are obtained from *Agrobacterium rhizogenes* and have genetic stability and high biosynthetic potential. *Withania somnifera* and *Rauwolfia serpentine* are a few among them (Singh et al., 2022; Srivastava et al., 2016).
- **Shoot/Root Organ Cultures:** Sustain site-specific biosynthesis, which generally yields more than undifferentiated cells (Kikowska et al., 2025; Sánchez-López *et al*., 2025).

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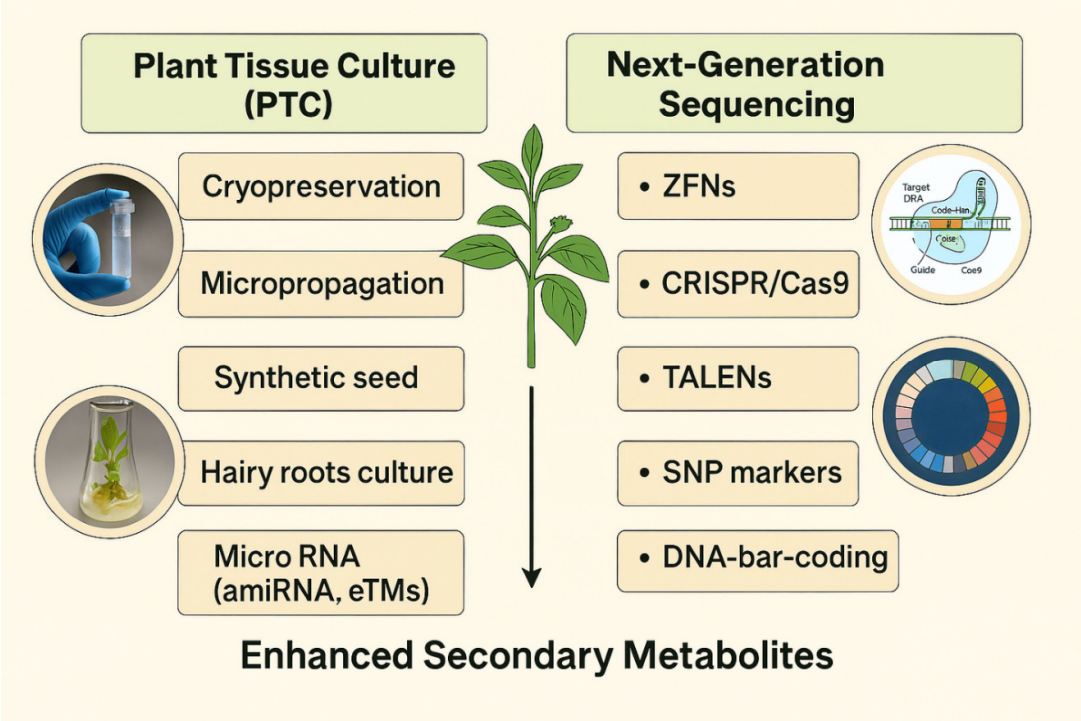

Figure 2. Innovative Pathways for Secondary Metabolite Enrichment in Medicinal Plants (Modified from Ghosh et al., 2024).

1.2. Elicitation Strategies

Elicitors are biotic or abiotic stress-inducing compounds that induce the formation of secondary metabolites by triggering plant defense mechanisms (Khan et al., 2025; Malu et al., 2025).

- **Biotic Elicitors:** Consist of fungal or bacterial cell wall materials e.g., chitosan (Javed *et al*., 2025), yeast extract (Lescano et al., 2025).
- **Abiotic Elicitors** Salicylic acid (Rithichai et al., 2024), jasmonic acid (Rasheed et al., 2017 silver nanoparticles (Bernela et al.,2023; Verma *et al*., 2024 and UV light are some of them.

These stimuli trigger cascades of signaling and transcription factors that upregulate the essential biosynthetic genes.

1.3. Precursor Feeding

The major metabolic precursors as Carbohydrates (Mukherjee et al., 2025) serve as basic inputs for the plant's biosynthesis of varying secondary metabolites (Figure 3).

Supplementation of biosynthetic precursors on cultures, e.g., shikimic acid, mevolunic acid, phenylalanine (Delfi et al., 2025) has been proven to boost the yields of metabolites by controlling the Pathways of the metabolites (Figure 3).

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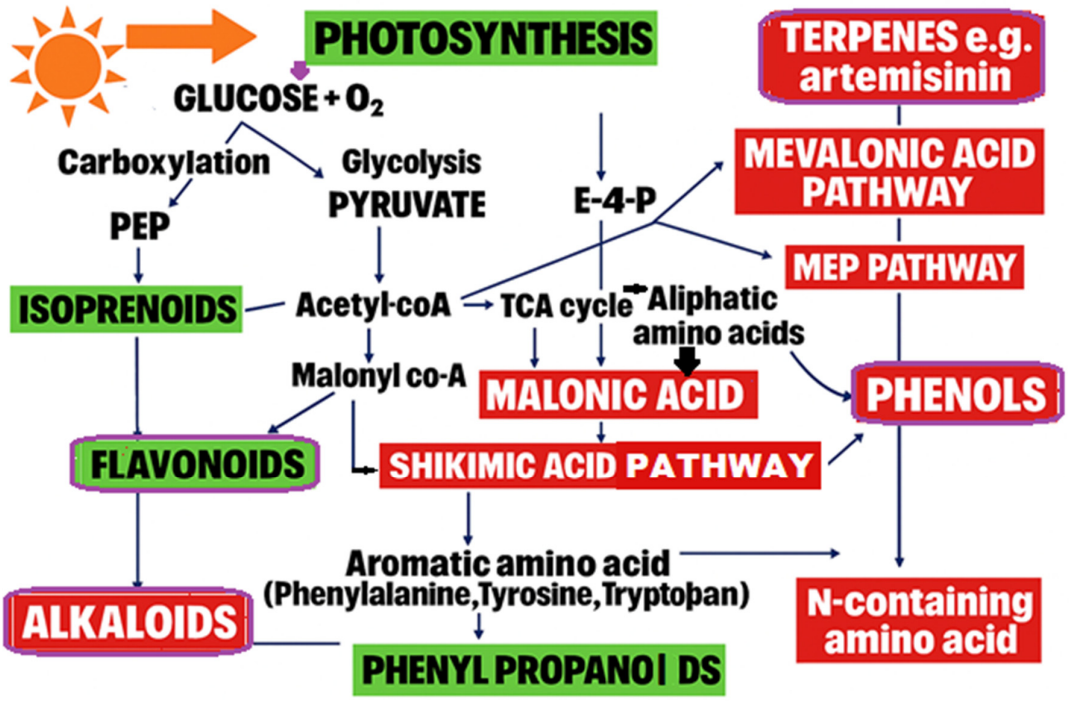

Figure 3. Flowchart of Plant Secondary Metabolite Biosynthetic Pathways (Modified from Kajla et al., 2023).

1.4. Metabolic

Metabolic refers to the alteration of genes that code for key enzymes in a pathway. Other approaches that have been used are:

- Overexpression of pathway-specific genes (e.g., PAL, CHS). Research has also been found to reveal that Salicylic acid and Gibberellic acid induce the expression of the phenylalanine ammonia-lyase (PAL) synthesis genes responsible for an increase in phenolics (e.g., rosmarinic acid) and falvanoid as well in *Salvia officinalis* (Moreira et al., 2022).
- Silencing of competitive pathways. The sterol pathway is a competitive pathway of artemisinin biosynthesis in *A. annua*. Four competitive branch pathway genes β-caryophyllene synthase gene (CPS), *β-farnesene synthase* gene (BFS), *germacrene A synthase* gene (GAS) and SQS were further down-regulated independently by the antisense method in *A. annua*. The content of artemisinin and dihydroartemisinic acid (DHAA) were increased significantly in different transgenic lines.
- Engineering transcription factors to co-upregulate multiple genes (Shi et al., 2024) (Figure 5).

Synthetic biology tools like CRISPR/Cas and synthetic promoters provide unadulterated control of metabolite flux (Gao et al., 2025) but along synthetic promoters, offer precise control over metabolite flux as GABA, with hypotensive properties enhanced in Tomato fruit and rice grains using this technology (Das et al., 2024)

1.5. Omics and Systems Biology Approaches

Multiomics integration i.e., Genomics, Transcriptomics, proteomics, and metabolomics give information about the regulatory networks (Figure 4) of secondary metabolism (**Karalija et al.,** 2025; Wu et al., 2025).

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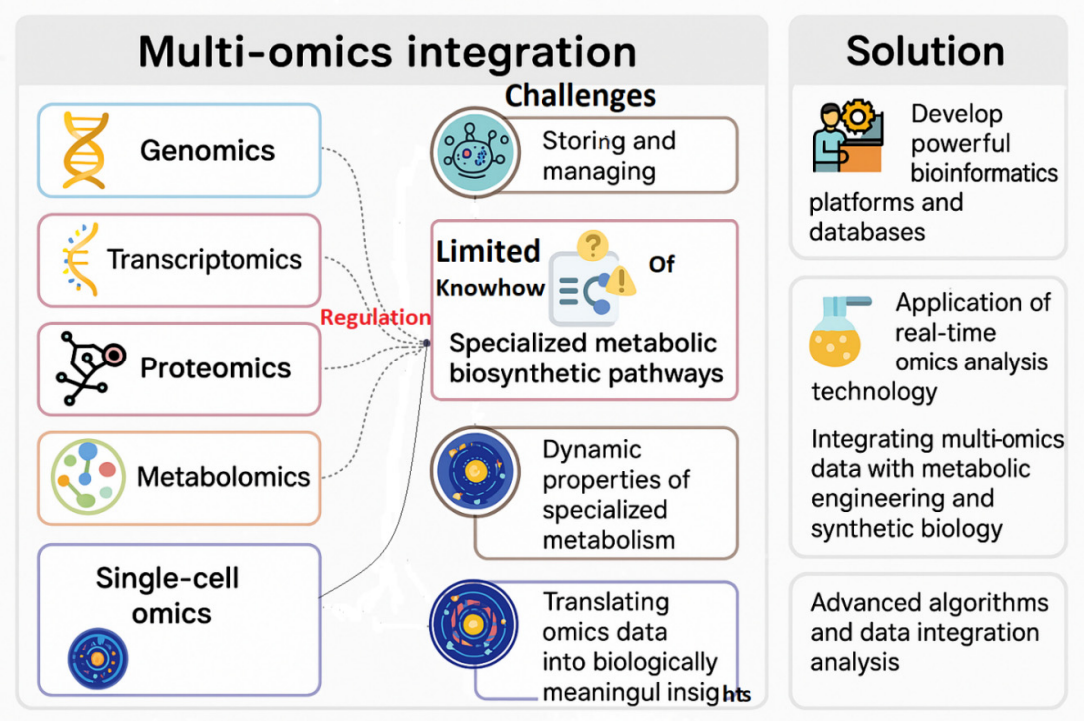

Figure 4. Multi-Omics integration in Medicinal Plants Metabolic Pathways, Challenges and Solutions (Modified from Wang et al., 2024).

This gives a picture of how the confluence of genomics, transcriptomics, proteomics, metabolomics, and single-cell omics allows deciphering and control of specialized metabolic processes in medicinal plants. Despite integration offering a general view of secondary metabolite biosynthesis, primary bottlenecks include handling data, paucity of pathway information, dynamic nature of metabolism, and impossibility of easy translation of complicated information. To counter these, solutions include high-end bioinformatics platforms, real-time omics techniques, synthetic biology integration, and high-performance data analysis software. In all, there is a need for multi-omics integration to enhance medicinal compound discovery and production (Liu et al., 2025b).

Multi-omics data integration facilitates:

- Selection of critical metabolic bottlenecks.
- Marker-assisted selection for cell lines with high yields.
- In silico modeling of biosynthetic networks for targeted intervention.

1.6. Limitations and Challenges

- Somaclonal variation in long-term cultures.
- Problems of scalability for commercial cultivation.
- Regulatory problems with genetically modified products.
- Limited knowledge of intricate metabolic controls in non-model plants (Dsouza et al., 2025; **Karalija et al.,** 2025; Singh et al., 2025).

2. Methodology

We reviewed using the systematic literature analysis approach to gather, analyze, and synthesize ongoing research on biotechnological interventions in the increase of secondary metabolite production in medicinal plants. The steps involved in the methodology were:

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2.1. Literature Search Strategy

A comprehensive literature search of peer-reviewed research papers, review articles, and conference papers was carried out using the following databases:

- **PubMed**
- **ScienceDirect**
- **Scopus**
- **Google Scholar**
- **SpringerLink**

The search covered journals from the period January 2010 to July 2025 but accentuating recent and most impactful developments post-2015. One article citation from bioRxiv repository on Artemsia database for revealing mul-tiomics research secrets was included (https://artemisia-db.com/).

2.2. Search Terms and Keywords

For seeking relevant articles, Boolean combinations of the following keywords were utilized:

- "Secondary metabolites"
- "Medicinal plants"
- "Plant tissue culture"
- "Hairy root culture"
- "Elicitation"
- "Metabolic engineering"
- "Synthetic biology"
- "Biotechnological enhancement"
- "Plant-derived compounds"
- "Omics approaches in plants"

2.3. Inclusion and Exclusion Criteria

Inclusion Criteria:

- Articles published in the English language.
- functor web Studies that report experimental or review data on biotechnological enhancement of secondary metabolites.
- functor web Research on plant in vitro culture methods (e.g., callus, hairy root, suspension culture).
- functor web Articles discussing elicitor-based, molecular, or omics-level intervention.

Exclusion Criteria:

- Studies not focused on medicinal plants.
- Articles with poor methodological description.
- Descriptions of primary metabolites or non-target plant species.
- Articles in non-peer-reviewed journals or without available full texts.

2.4. Organization and Data Extraction

Relevant data were categorized and grouped based on:

- Type of biotechnological intervention (e.g., elicitation, genetic modification, tissue culture)
- Plant species studied
- Targeted enhanced metabolite(s)
- Mechanism or pathway targeted
- Experimental outcome and yield improvements

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Tables and figures were prepared to integrate interventions, mark interesting studies, and compare improvement strategies across plant systems.

2.5. Review Process and Quality Evaluation

Each article was critically examined for:

- Scientific quality and reproducibility
- Novelty in methodology
- Relevance to upgrading of pharmaceutically important compounds

Only well-documented, high-quality studies were used to give the review reliability.

2.6. Synthesis of Results

The information was synthesised thematically and classified into:

1. In vitro tissue culture techniques
2. Elicitor-based improvement
3. Synthetic biology and metabolic engineering
4. Indirect tissue culture strategies (miRNA, Ploidy)
5. Omics and systems biology platforms

The final draft of the review was prepared to paint a homogeneous and coherent picture of the existing status and future prognosis of biotechnological interventions in this sector.

3. Results

A meta-analysis of more than 100 peer-reviewed articles demonstrated a broad range of biotechnological interventions effectively used to improve secondary metabolite yield in medicinal plants. The results have been grouped into major intervention strategies: in vitro tissue culture, elicitation, metabolic engineering, and omics-based strategies. The rundown of significant outcomes is given below.

3.1. Tissue Culture-Based Enhancement

Several plant species showed enhanced metabolite production through callus, suspension, or hairy root cultures (Table 1):

Table 1. Biotechnological In Vitro Culture Strategies and Approaches for Enhanced Secondary Metabolite Production in Medicinal Plants.

Plant Species	Culture Type	Target Metabolite	Yield Increase	Reference
Artemisia annua	Organ culture	Artemisinin	7.8 fold	Al-Khayri *et al*., 2022
Rauwolfia serpentina	Callus culture	Reserpine	2 fold	Gantet *et al*., 2023
Taxus brevifolia and *Taxus cuspidata*	Cell suspension	Paclitaxel	Commercial large scale	Fett-Neto & DiCosmo, 2020; Zhao *et al*., 2023
Withania somnifera	Hairy roots	Withanolides	11.49 fold	Haider and Gosh, 2024

3.2. Elicitor-Mediated Enhancement

Elicitors like jasmonic acid (JA), salicylic acid (SA), yeast extract, and silver nanoparticles (AgNPs) showed an excellent effect on the secondary metabolite biosynthesis (Table 2).

Table 2. Influence of Different Elicitors on Secondary Metabolite Production in Selected Medicinal Plants.

Elicitor Type	Plant Model	Metabolite	Observed Effect	Reference
AgNPs	*Achillea millefolium*	Essential oil	2.3× increase	Lala et al., 2021

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Cellulase	*Glycyrrhiza glabra*	Glycyrrhizin	8.6× increase	Srivastava *et al*., 2019
Jasmonic Acid	*Catharanthus roseus*	Vinblastine, Vincristine	0.6× increase	Rasheed *et al*., 2017
Salicylic Acid	*Ocimum sanctum*	Eugenol	282.96× increase	Rithichai *et al*., 2024
Yeast Extract	*Panax ginseng*	Ginsenosides	1.57× increase	Kochan *et al*., 2017

3.3. Genetic Modification and Metabolic Engineering

Metabolic pathway engineering by overexpression of dominant biosynthetic genes and inhibition of unwanted pathways resulted in remarkable enhancement of metabolite accumulation (Table 3):

Table 3. Genetic and Signaling-Based Approaches for the Improvement of Secondary Metabolite Production in Medicinal Plants.

Modification Strategy	Plant	Gene Targeted	Result	Reference
3-hydroxy-3-methylglutaryl coenzyme and amorpha-4,11-diene synthase coexpression	*Artemisia annua*	*HMGR* and *ADS* genes	↑Artemisinin 8.65× increase	Zhao et al., 2022
ADS-FPPS enzymes co-expression	*Artemisia annua*	*ADS-FPPS* genes	↑Artemisinin 2.6× increase	Zhao et al., 2022
Co-expression of TFs ORCA3 and MYC2	*Catharanthus roseus*	Transcriptional regulators	↑ TIA Biosynthesis as Vincristine and ajmalicine	Paul *et al*., 2017
FPPS, *CYP71AV1* and *CPR* enzymes co-expression	*Artemisia annua*	*FPPS*, *CYP71AV1* and *CPR* genes	↑Artemisinin 3.6× increase	Zhao et al., 2022
Overexpression of CHS	*Glycyrrhizia uralensis*	Chalcone synthase	↑ Flavonoid content	Yin *et al*., 2020
salicylic acid/H_2O_2	*Capsicum annuum*	Phenylalanine ammonia-lyase	↑ Capsiate production	Zunun-Pérez *et al*., 2017

3.4. Indirect Methods of Plant Tissue Culture Use

3.4.1. MicroRNA (amiRNA, eTMs)

Plant microRNAs (miRNAs), a family of roughly 21-nucleotide-long small noncoding RNAs (ncRNAs), typically function as important regulators of their target genes by directing mRNA cleavage or translational repression (Jiang et al., 2021; Ghosh et al., 2024). In contrast, miRNAs are directly implicated in the biosynthesis regulation of Secondary metabolites like alkaloids, flavonoids, and terpenes that are responsible for the therapeutic activity (Yang et al., 2025).

3.4.2. Ploidy Engineering

In medicinal plants, secondary metabolite production per unit of biomass has vast economic importance. Therefore, doubling the genome of cells promotes genome multiplication, protein synthesis increase, and secondary metabolite yield increase (Ghosh et al., 2024).

3.5. Omics and Systems Biology Contributions

Omics data integration (transcriptomics, proteomics, metabolomics) gave information about pathway regulation e.g., biosynthesis of artemsinin (Shi et al., 2024) in *A. annua* (Figure 5) and revealed new targets for intervention. The use of CRISPR/Cas9 and other gene-editing technologies may enable the metabolic engineering of medicinal plants for boosting specialized metabolite production Integrated multi-omics offers a promising route for boosting our comprehension of

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interactions among genes, proteins, and metabolites, potentially solving for new specialized metabolites of therapeutic interest (Shi et al., 2024; Wang *et al*., 2024).

4. Discussion

The improvement of secondary metabolite production in medicinal plants is a center of attention in plant biotechnology because of the worldwide demand for natural therapeutic products. The paper has highlighted the wide range of biotechnological interventions ranging from multiomics inetgartion with in vitro culture systems to elicitor applications and genetic engineering interventions that have been proved effective in improving yield and quality of bioactive compounds **(**Karalija et al., 2025; Shi et al., 2024; Wang *et al*., 2024).

In vitro culture systems, such as callus culture, cell suspension, hairy roots, and organ cultures, provide controlled and scalable systems for metabolite production, irrespective of environmental oscillations. Other studies, such as those on Withania somnifera, Artemisia annua, and Taxus spp., have recorded significant fold increases in the accumulation of secondary metabolites under optimized culture conditions.These platforms are also useful assets in biosynthetic pathway and regulatory process research (Al-Khayri et al., 22; Fett-Neto & DiCosmo, 2020; Haider & Ghosh, 2024; Zhao et al., 2023).

The use of elicitors, such as jasmonic acid, salicylic acid, and yeast extract, has been especially effective in eliciting stress-like reactions in plant cells, thus upregulating the genes of interest associated with secondary metabolite biosynthesis. For instance, the elicitation of Catharanthus roseus with jasmonic acid has been reported to promote vincristine and vinblastine production, both of which possess pivotal anticancer activity. The above results highlight the prospect of elicitation as an affordable and scalable means of boosting metabolite production (Lala et al., 2021; Kochan *et al*., 2017; Rasheed *et al*., 2017; Rithichai *et al*., 2024).

Genetic manipulation has also propagated this discipline by allowing for targeted manipulation of biosynthetic genes and regulatory transcription factors. Methods such as overexpression of chalcone synthase in Glycyrrhiza uralensis or co-expression of ORCA3 and MYC2 transcription factors in Catharanthus roseus have led to the immense upregulation of flavonoids and terpenoid indole alkaloids, respectively. These therapies not only increase metabolite quantity but also increase the reproducibility and stability of the production (Paul *et al*., 2017; Yin *et al*., 2020; Zhao et al., 2022; Zunun-Pérez *et al*., 2017).

4.1. Refinement of Tissue Culture Based

The data presented (Table 1) exhibit very clearly the potential of biotechnological in vitro culture methods to augment greatly the yield of valuable secondary metabolites from medicinal plants. A major antimalarial metabolite, artemisinin, was increased 7.8 times by organ cultures of *Artemisia annua*, demonstrating the possibility of tissue-specific growth under controlled conditions for metabolite enhancement. Concurrently, callus culture of *Rauwolfia serpentina* reflected a 2-fold enhancement in reserpine content, demonstrating the capability of dedifferentiated plant tissues to serve as biofactories for alkaloid production. Cell suspension culture of *Taxus brevifolia* and *T. cuspidata* are notable in having reached levels of commercial paclitaxel production, an anticancer drug of efficacy, demonstrating the scalability of this technology. Besides, *Withania somnifera* hairy root cultures established through Agrobacterium rhizogenes-mediated transformation yielded a remarkably 11.49-fold greater withanolide accumulation, underscoring the relevance of *Agrobacterium rhizogenes*-mediated transformation in high-yielding and stable metabolite biosynthesis platforms. Together, these examples support the applicability of in vitro biotechnologies as effective and sustainable alternative means to classical harvesting from natural plant populations for conservation and development of pharmaceutical compounds (Karalija et al., 2025; Shi et al., 2024; Wang *et al*., 2024).

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4.2. Elicitor Mediated Enhancement

The results (Table 2) reveal the heterogeneous and high-order impacts of various elicitors on inducing secondary metabolite biosynthesis in medicinally important plants, exemplifying a planned strategy towards plant biotechnology. Salicylic acid of the tested elicitors showed a very high stimulation with a 282.96-fold increase in eugenol production in *Ocimum sanctum* (Rithichai *et al*., 2024), suggesting intensive activation of the phenylpropanoid pathway. In the same way, the biotic elicitor cellulase caused an 8.6-fold enhancement of glycyrrhizin content in *Glycyrrhiza glabra*, showing its function in induction of metabolic flow possibly through cell wall breakdown and increased precursor supply (Srivastava *et al*., 2019). Silver nanoparticles (AgNPs), as abiotic nanomaterial-based elicitors, enhanced essential oil production by 2.3 times in *Achillea millefolium*, demonstrating their role in metabolite accumulation in response to oxidative stress (Lala *et al*., 2021). Conversely, yeast extract, an intricate biotic elicitor, increased ginsenosides moderately by 1.57-fold in *Panax ginseng* (Kochan *et al*., 2017), pointing to its impact on terpenoid biosynthetic pathways. Surprisingly, jasmonic acid, well known for its role in secondary metabolism regulation, modestly enhanced vinblastine and vincristine content in *Catharanthus roseus* by only 0.6× (Rasheed *et al*., 2017), indicating that the efficiency of elicitors tends to be species- and pathway-specific. These observations individually highlight the promise of elicitation strategies to maximize metabolite productivity, albeit with effectiveness being strongly associated with the plant species, elicitor dose, and exposure regime (Lala et al., 2021; Kochan *et al*., 2017; Rasheed *et al*., 2017; Rithichai *et al*., 2024).

4.3. Metabolic Engineering and Genetic Modification

The information (Table 3) reveals a variety of genetic and signaling-driven engineering tools aimed at the enhancement of secondary metabolite production in medicinal plants, emphasizing the precision and efficacy of manipulation of metabolic pathways. Among the key strategies is gene co-expression, e.g., HMGR (3-hydroxy-3-methylglutaryl-CoA reductase) and ADS (amorpha-4,11-diene synthase) overexpression simultaneously in *Artemisia annua*, which showed 8.65-fold increased artemisinin yield (Zhao *et al*., 2022). This notable enhancement reflects precursor supply (via HMGR) and step-specific catalysis (via ADS) strategic enhancement in terpenoid biosynthesis.

Likewise, co-expression of ADS with FPPS (farnesyl diphosphate synthase) and FPPS with CYP71AV1(cytochrome P450 monooxygenase) and CPR (cytochrome P450 reductase) also augmented artemisinin yields 2.6× and 3.6×, respectively, demonstrating the significance of synergistically enhancing both precursor production and downstream oxidative processes (Zhao *et al*., 2022).

In Catharanthus roseus, co-expression of transcription factors MYC2 and ORCA3 activated the terpenoid indole alkaloid (TIA) pathway, resulting in enhanced biosynthesis of anticancer metabolites like vincristine and ajmalicine (Paul *et al*., 2017). This is a sign of the potential of the transcription factors to control the various biosynthetic genes globally and therefore are hopeful targets for general metabolic enhancement.

As a second example, CHS overexpression in Glycyrrhizia uralensis activated flavonoid production, demonstrating the efficacy of rate-limiting enzyme targeting within the phenylpropanoid pathway (Yin *et al*., 2020). Furthermore, treatment with salicylic acid and hydrogen peroxide in Capsicum annuum elicited phenylalanine ammonia-lyase (PAL) activity, leading to enhanced levels of capsiate, suggesting that signaling molecules could regulate defense pathways to promote metabolite accumulation (Paul *et al*., 2017; Yin *et al*., 2020; Zhao *et al*., 2022; Zúnún-Pérez *et al*., 2017).

Together, these findings demonstrate that gene stacking, transcription factor manipulation, and induction of the signaling are robust pathway engineering tools that can be modulated to overproduce particular kinds of secondary metabolites in a species- and pathway-dependent manner.

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4.4. Ploidy Engineering

The information (Table 4) illustrates the effect of agents inducing polyploidy mainly colchicine and oryzalin on the production of secondary metabolites in medicinal plants. Polyploidy, through doubling the chromosomes, tends to result in increased gene dosage, increased nuclear content, and metabolic changes, which can activate biosynthetic pathways. For instance, induction of tetraploidy in *Ajuga reptans* with oryzalin increased 20-hydroxecdysone production, a major phytoecdysteroid (Navrátilová *et al*., 2022; Svecarova *et al*., 2019). Likewise, colchicine treatment doubled *Catharanthus roseus* anticancer alkaloid vincristine yields (Fathimunnissa, 2011), and increased 4-fold bacoside deposition in *Bacopa monnieri* (Kharde *et al*., 2017), which indicates that autotetraploidy can immensely increase secondary metabolite yields in certain species. However, not all the events were successful *Artemisia annua* and *Echinacea purpurea* showed suboptimal or slight increments in artemisinin, cichoric acid, and chlorogenic acid after colchicine-induced tetraploidy (Lin *et al*., 2011; Abdoli *et al*., 2013), It described the response as species-specific and metabolite-specific. Further, colchicine-induced polyploidy in Linum album increased podophyllotoxin production by 1.39× (Javadian *et al*., 2017), and, thus indicated potential in the optimization of lignan pathways. These findings in general indicate that although polyploidy is a useful approach for metabolite improvement, its efficacy is plant genotype-, target compound-, and regulatory network complexity-dependent **(**Navrátilová et al., 2022; Svecarova et al., 2019; Fathimunnissa, 2011; Kharde et al., 2017; Lin et al., 2011; Abdoli et al., 2013; Javadian et al., 2017).

Table 4. Impact of Polyploidy-Inducing Agents on Medicinal Plant Secondary Metabolite Yield.

Agent	Plant Model	Mechanism	Metabolite	Observed Effect	Reference
Oryzalin	*Ajuga reptans*	Tetraploidy	20-hydroxecdysone	Increased content	Navrátilová et al., 2022; Svecarova et al., 2019
Colchicine	*Catharanthus roseus*	Autoploidy (chromosome doubling)	vincristine	2× increase	Fathimunnissa, 2011
Colchicine	*Bacopa monnieri*	Autoploidy (diploidy)	bacoside	4× increase	Kharde et al., 2017
Colchicine	*Artemisia annua*	Tetraploidy	artemisinin	0.7× increase	Lin et al., 2011
Colchicine	*Echinacea purpurea*	Tetraploidy	cichoric acid and chlorogenic acid	0.45× and 0.71× increase respectively	Abdoli et al., 2013
Colchicine	*Linum album*	Polyploidy	podophyllotoxin	1.39× increase	Javadian et al., 2017

4.5. Engineering Strategies to Enhancing Artemisinin Synthesis in Artemisia annua

Artemisinin, a sesquiterpene lactone that has been purified from Artemisia annua, is in huge market demand because of its strong antimalarial effect (Yuan *et al*., 2023). The integrated model for the biosynthesis pathway of artemisinin in Artemisia annua (Figure 5), showing the ubiquitous nature of transcription factors (TFs) to regulate the crucial enzymic steps.The biosynthesis is started by photosynthesis-driven glucose production that is diverted along the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways to produce the precursors IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate). These are then decreased by farnesyl diphosphate synthase (FPS) to become FPP (farnesyl diphosphate) the universal substrate of sesquiterpenoids (Hassani *et al*., 2020).

The first committed step of artemisinin biosynthesis is catalyzed by *amorphadiene synthase* (*ADS*), which produces amorpha-4,11-diene from FPP. The intermediate then goes through a series of

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oxidative and reductive conversions by cytochrome P450 enzyme *CYP71AV1*, which converts it sequentially into artemisinic alcohol, artemisinic aldehyde, and ultimately to dihydroartemisinic aldehyde by the action of *DBR2* (*double bond reductase 2*). Secondary conversion by *ALDH1* (*aldehyde dehydrogenase 1*) produces dihydroartemisinic acid, which, through spontaneous photooxidation, produces artemisinin in glandular trichomes. Four genes in artemisinin-specific biosynthetic pathway namely *ADS, CYP71AV1, DBR2 and ALDH1* were expressed at greater levels in MeJasmonic acid/JA treatment (Hassani *et al*., 2020; Yan *et al*., 2017; Xie *et al*., 2021; Xiang *et al*., 2015).

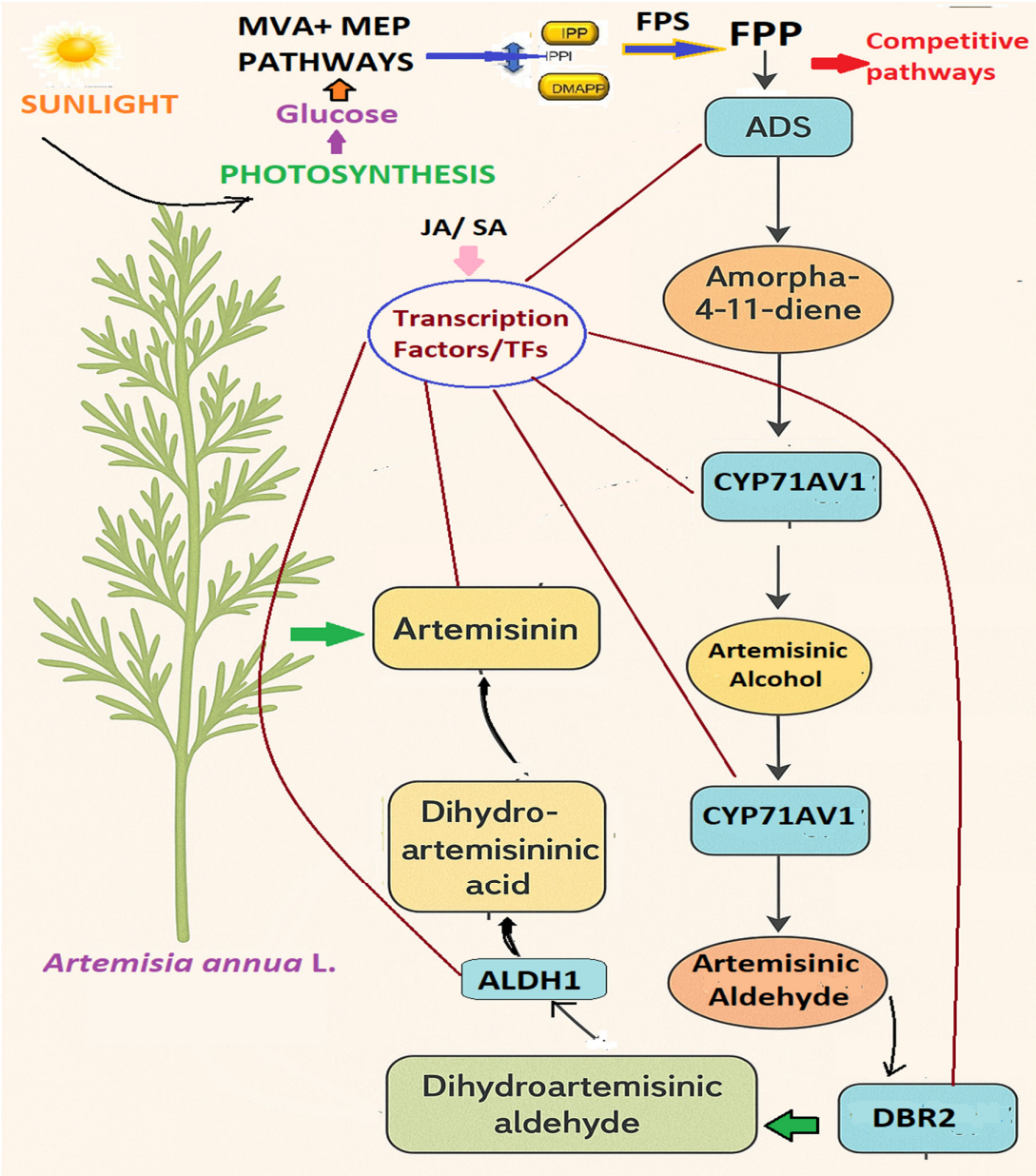

Figure 5. Simplified Integrating of Transcription Factor Dynamics into Artemisinin Biosynthesis Models in *A. annua* (Modified from Shi et al., 2024) Note: The blue boxes show enzymes, JA=Jasmonic acid and SA= Salicylic acid, ADS= amorpha-4,11-diene synthase, ALDH1= aldehyde dehydrogenase 1, CYP71AV1= cytochrome P450-dependent hydroxylase, DBR2=double bond reductase 2, DMAPP= dimethylallyl diphosphate, FPP= farnesyl diphosphate, FPS= farnesyl diphosphate synthase, IP=, isopentenyl diphosphate, IPPI= isopentenyl diphosphate isomerase, MVA= mevalonate; MEP, methyl-D-erythritol 4-phosphate. Notably, this number highlights the

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pivotal regulatory function of transcription factors, portrayed as red arrows that affect almost every enzymatic step.

TFs regulate the level of structural genes for enzymes like ADS, CYP71AV1, DBR2, and ALDH1, thus fine-tuning the metabolic flow in the pathway.

Environmental conditions such as sunlight also have a crucial role, both in initiating photosynthesis and in the terminal non-enzymatic photooxidation step to produce artemisinin. The research result (Figure 6) of Yuan *et al*., (2023) illustrates that Jasmonic acid (JA) is a major elicitor, triggering transcription factors signaling cascade like AabHLH112, AaERF1, and AabHLH113, which indirectly regulate the expression of core biosynthetic genes like *ADS, CYP71AV1, DBR2, and ALDH1* upregulate encoded upstream enzymes. Jasmonic acid induces the activation of transcription factors AabHLH112 and AaERF1, which induce the upregulation of upstream *ADS* and *CYP71AV1* enzymes catalyzing early steps from farnesyl diphosphate to artemisinic aldehyde. At the same time, AabHLH112 interact AabHLH113, which control *DBR2* and *ALDH1* enzymes catalyzing conversion of artemisinic aldehyde to dihydroartemisinic acid, a direct intermediate of artemisinin. This two-pathway convergence emphasizes the transcriptional control sites necessary for metabolic engineering for increasing The biosynthesis of artemisinin A useful antimalarial sesquiterpene lactone is a highly regulated Jasmonic acid(JA)-responsive metabolic process in *Artemisia annua* (Rasheed *et al*., 2017; Xiang *et al*.,2019; Yuan *et al*., 2025).

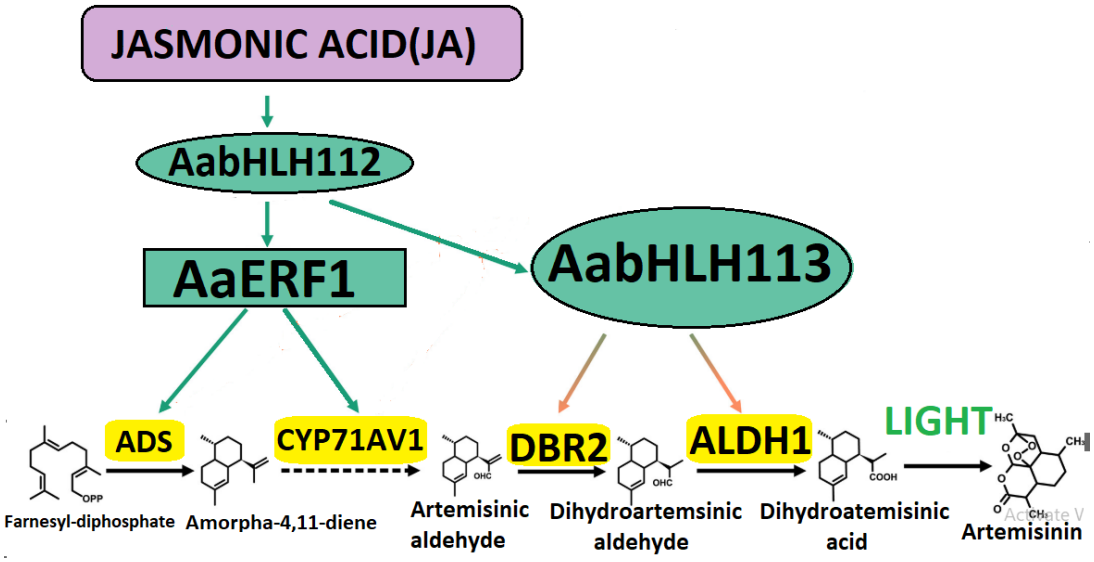

Figure 6. Transcriptional Regulation of Artemisinin Biosynthesis via JA Signaling Pathways in *Artemisia annua* (Modified from Yuan et al., 2023) Note: The large green boxes show Transcription Factors/TFs,Yellow boxes= Enzymes, JA=Jasmonic acid, ADS= amorpha-4,11-diene synthase, CYP71AV1= cytochrome P450-dependent hydroxylase, , DBR2=double bond reductase 2, ALDH1= aldehyde dehydrogenase 1.

These differential patterns reveal spatial regulation of artemisinin pathway genes and can guide tissue-targeted metabolic engineering. To further explore gene annotations, expression matrices, and visualization tools, the Artemisia Database is an exhaustive platform for transcriptomic analysis and functional genomics.

These differential patterns based on Artmesia database heatmap (Figure 7) reflect the spatial regulation of artemisinin pathway genes in five organs of *Artemisia annua* (Leaf, Petiole, Flower, Root, Stem) and have the potential to inform tissue-targeted metabolic engineering strategies. Of particular note, mikado.chr4G1338 (*ADS*) and mikado.chr3G1536 (*CYP71AV1*) are found to have maximum expression intensities in leaf and flower tissues, followed by stem and petiole, as highlighted by the red coloration. The heatmap scale indicates expression values from 0 (dark blue, lowest) to 3 (red,

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highest). The Gene IDs and their respective gene names are mikado.chr4G1338=*ADS*, mikado.chr3G1536=*CYP71AV1*, mikado.chr2G1956=*DBR2*, and mikado.chr8G609=*ALDH1*. For more exploration of gene annotations, expression matrices, and visualization tools, the Artemisia Database offers an extensive platform for transcriptomic analysis and functional genomics (https://artemisia-db.com/).

Therefore, this combined model not only traces out the metabolic pathway but also captures the intricate interaction between metabolic enzymes and transcriptional regulation, providing a useful platform for metabolic engineering approaches to boost artemisinin productivity in *A. annua (Kumari* et al., *2025;Taheri* et al., *2025)*.

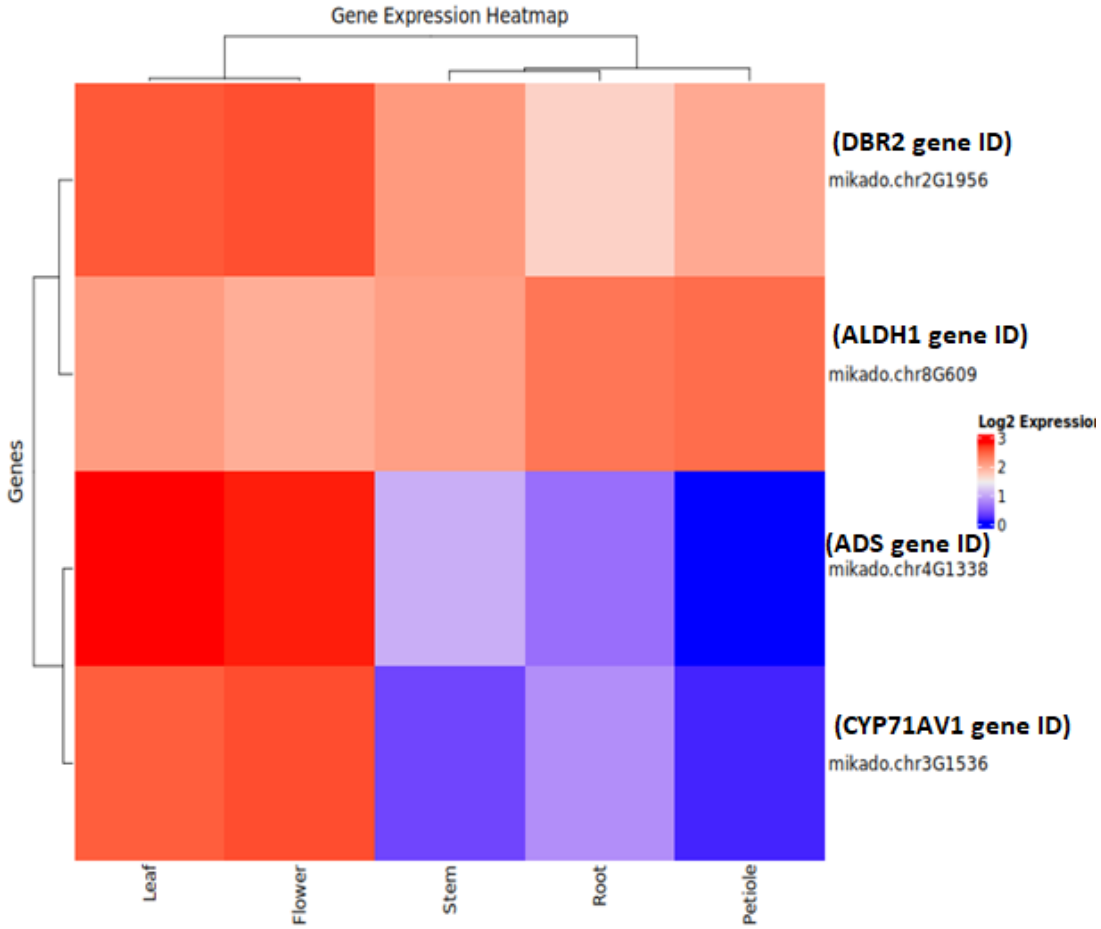

Figure 7. The Artemisinin database output analysis of **Tissue-Specific Gene Expression Heatmap Landscapes of Artemisinin Biosynthetic Genes in *Artemisia annua*: Insights from Spatial Transcriptomics and Functional Genomics.** Note: Gene IDs and the consecutive genes names are mikado.chr4G1338=ADS, mikado.chr3G1536=CYP71AV1, mikado.chr2G1956=DBR2, mikado.chr8G609=ALDH1. (https://artemisia-db.com/).

5. Future Prospects

The future of the production of secondary metabolites from medicinal plants is in precision bioengineering, multi-omics-driven design, and scalable and sustainable production platforms. With responsible innovation and interdisciplinary collaboration, there is the potential for transforming natural product biosynthesis to respond to the increasing global need for safe, effective, and affordable plant-derived therapeutics. Future approaches such as AI-assisted metabolic flux analysis, genome editing, and machine-learning models have the potential to revolutionize secondary

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metabolite enhancement. Combination with vertical farming and bioreactors can result in commercial-scale production with low environmental impact.

6. Conclusions

Biotechnological methods offer not just a practical replacement for conventional extraction techniques but also an environmentally friendly and scalable means to fulfilling international needs for plant-based therapeutics. DNA Biotechnological applications offer promising, sustainable, and scalable methods towards enhancing secondary metabolites in medicinal plants. Continued advancement of molecular biology, systems biology, and synthetic biology will give the foundation for efficient production of such vital compounds to meet global pharmaceutical and nutraceutical demands. Additional interdisciplinarity research and technological fusion will be the key to fully exploiting the potential of medicinal plants during the 21st century.

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