

# Targeted Biotechnological Interventions for High-Value Secondary Metabolite Production in Medicinal Plants

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## Abstract

Due to their abundance of secondary metabolites with pharmacological activity, medicinal plants have long been a cornerstone of both conventional and contemporary healthcare systems. However, the amount of these beneficial molecules produced naturally is often modest, irregular, and significantly influenced by environmental factors. In response, recent advancements in plant biotechnology offer promising solutions for enhancing the yield and quality of secondary metabolites in a controlled and sustainable manner. This paper examines the range of biotechnological tools and techniques utilised to enhance secondary metabolite biosynthesis in medicinal plants, including plant tissue culture, elicitation, hairy root culture, metabolic engineering, and CRISPR/Cas9 genome editing. To highlight the biological importance and potential therapeutic uses of secondary metabolites, we first categorise them into groups such as terpenoids, alkaloids and phenolics. After that, we explore specific biotechnological approaches that alter these pathways, going over how metabolic flux can be impacted by external elicitors, precursor feeding, and gene overexpression. We present a thorough examination of the present situation in this research and the future potential of biotechnological approaches for enhancing the ability of medicinal plants to produce secondary metabolites. We focus on several key tactics, including the use of elicitors, genetic and metabolic engineering, and tissue culture-based production systems. We also highlight several case studies in detail, featuring medicinal plants such as *Withania somnifera*, *Artemisia annua*, and *Panax ginseng*. These examples demonstrate real-world applications of biotechnology in boosting the production of

withanolides, artemisinin, and ginsenosides. By doing so, we aim to highlight how plant biotechnology can be used sustainably for pharmaceutical development, medicinal plant research, and conservation. This paper offers a thorough summary of how plant biotechnology is revolutionising the field of natural product research. These developments help preserve the biodiversity of medicinal plants while also enhancing access to life-saving plant-based medications by fusing traditional knowledge with contemporary research.

**Keywords:** Secondary metabolites, Medicinal plants, Metabolic engineering, Plant biotechnology, Omics approaches, Secondary metabolites, Pharmaceutical compounds

## **Introduction**

Numerous chemical compounds are produced by plants, which are broadly categorised into primary and secondary metabolites. Proteins, lipids, and carbohydrates are examples of primary metabolites that are necessary for plant growth and development. Secondary metabolites are non-essential compounds that serve ecological functions, including defence against herbivores and pathogens, protection from UV radiation, and attraction of pollinators (Verpoorte, 1998; Crozier et al., 2006). Despite their secondary nature, these compounds are of immense medicinal value and form the chemical basis of many modern drugs and traditional remedies. Key groups of secondary metabolites include alkaloids (e.g., morphine, vincristine), terpenoids (e.g., artemisinin, taxol), flavonoids (e.g., quercetin), and phenolic compounds (e.g., curcumin), many of which exhibit antimicrobial, anticancer, anti-inflammatory, antioxidant, and antimalarial activities (Ghosh, 2016; Wink, 2010). In the pharmaceutical and cosmetic industries, secondary metabolites can be utilised as raw materials. In the food industry, they can be used as food additives and to preserve crops in agriculture. Because secondary metabolites are so important, a lot of research has been done on their production, including possibilities connected to gene modification and biosynthesis for satisfying the needs of high-value agroecosystems. Insecticides (laurine, chlorobutanol, etc.) (Zhang et al., 2017), hallucinogens (morphine, scopolamine, tetrahydrocannabinol, etc.) (Batool et al., 2020), therapeutic agents (codeine, atropine, cardenolide, etc.) (Rashid et al., 2021), antioxidants (carsonic acid, rosemary oil, etc.) (Sahoo et al., 2022), and fragrances (capsaicin, vanillin, mustard oils, etc.) (Zachariah & Leela, 2018). Nevertheless, the natural production of these bioactive metabolites is often sluggish, tissue-based, and dependent on several factors, including the age of the plant, developmental phase, seasonal, and environmental stress (Ravishankar & Chattopadhyay, 2020). Additionally, the low content of these metabolites in nature, coupled with excessive harvesting and destruction of habitats, presents a significant challenge

to the sustainable production and conservation of medicinal plant resources (Singh et al., 2021). Of these, plant cell and organ cultures—notably hairy root cultures—have proved highly useful for the stable and scalable production of a range of value-added secondary metabolites (Gutierrez-Valdes et al., 2020). Apart from that, the complementarity with omics technologies (transcriptomics, genomics, proteomics, and metabolomics) significantly increased understanding of biosynthetic networks and facilitated the rational design of metabolic engineering strategies (Tiwari et al., 2022).

Biotechnological strategies offer effective means to transcend these disadvantages by maximising the in vitro production of secondary metabolites in reproducible and controlled conditions. Biotechnological methods provide strong countermeasures to these limitations by improving the in vitro manufacture of secondary metabolites under reproducible and controlled conditions. Techniques such as plant tissue culture, genetic transformation, elicitor treatment, metabolic pathway engineering, and synthetic biology enable the manipulation and optimisation of metabolite biosynthesis pathways (Zhao et al., 2019; Georgiev et al., 2009). In addition, the consolidation of omics technologies (transcriptomics, genomics, proteomics, and metabolomics) has greatly refined our understanding of biosynthetic networks as well as enabled the rational design of metabolic engineering strategies (Tiwari et al., 2022).

### Classification Of Secondary Metabolites

Terpenes, phenols, and nitrogen-containing compounds are the three chemically distinct categories into which plant secondary metabolites can be classified.

Lipids called terpenes are made from basic glycolysis intermediates or Acetyl-CoA.

Phenolic compounds are aromatic substances that are produced through either the Shikimic acid pathway or the Malonic acid pathway (Ramírez-Gómez et al., 2019).

Amino acids are the primary source of biosynthesis for nitrogen-containing secondary metabolites, including alkaloids.

**Table 1. Types of plant secondary metabolites**

| Type of Metabolite | Example(s)                     | Function in Plant                                  | Plant Source(s)                                                                            | Pharmaceutical Use                                                      | References                             |
|--------------------|--------------------------------|----------------------------------------------------|--------------------------------------------------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------|
| Alkaloids          | Morphine, Vincristine, Quinine | Defense against herbivores, antimicrobial activity | <i>Papaver somniferum</i> ,<br><i>Catharanthus roseus</i> ,<br><i>Cinchona officinalis</i> | Analgesic (morphine), anti-cancer (vincristine), antimalarial (quinine) | Dewick (2002); Verpoorte et al. (2000) |

|                       |                                          |                                                  |                                                                              |                                                                      |                                               |
|-----------------------|------------------------------------------|--------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------------|
| <b>Phenolics</b>      | Resveratrol, Flavonoids, Tannins         | UV protection, pathogen defense, structural role | <i>Vitis vinifera</i> , <i>Camellia sinensis</i> , <i>Terminalia chebula</i> | Antioxidant, anti-inflammatory, cardioprotective                     | Pandey & Rizvi (2009); Dixon & Paiva (1995)   |
| <b>Terpenoids</b>     | Artemisinin, Menthol, Taxol (Paclitaxel) | Attract pollinators, defense against pests       | <i>Artemisia annua</i> , <i>Mentha spp.</i> , <i>Taxus brevifolia</i>        | Antimalarial (artemisinin), anticancer (taxol), antiseptic (menthol) | Croteau et al. (2000); Tang et al. (2014)     |
| <b>Glycosides</b>     | Digoxin, Saponins, Salicin               | Defense, allelopathy                             | <i>Digitalis purpurea</i> , <i>Glycyrrhiza glabra</i> , <i>Salix alba</i>    | Cardiac stimulant (digoxin), anti-inflammatory, expectorant          | Hostettmann & Marston (1995); Harborne (1998) |
| <b>Saponins</b>       | Dioscin, Ginsenosides                    | Anti-herbivory, antimicrobial                    | <i>Dioscorea spp.</i> , <i>Panax ginseng</i>                                 | Anticancer, immunomodulatory, adaptogenic                            | Sparg et al. (2004); Christensen (2009)       |
| <b>Lignans</b>        | Podophyllotoxin                          | Defense, antioxidant activity                    | <i>Podophyllum hexandrum</i>                                                 | Precursor of anticancer drugs (etoposide)                            | Gordaliza et al. (2004)                       |
| <b>Coumarins</b>      | Umbelliferone, Scopoletin                | Antifungal, allelopathic                         | <i>Citrus spp.</i> , <i>Ruta graveolens</i>                                  | Anticoagulant, anti-inflammatory                                     | Bourgaud et al. (2006)                        |
| <b>Tannins</b>        | Catechins, Ellagitannins                 | Protection against predation and infection       | <i>Camellia sinensis</i> , <i>Punica granatum</i>                            | Antioxidant, antimicrobial, anticancer                               | Chung et al. (1998)                           |
| <b>Glucosinolates</b> | Sulforaphane                             | Herbivore deterrent                              | <i>Brassica oleracea</i> , <i>Raphanus sativus</i>                           | Anticancer (chemopreventive)                                         | Fahey et al. (2001)                           |

## Challenges in Natural Production and the Need for Biotechnological Approaches

Medicinal plants continue to be a stronghold of traditional medicine on account of their rich pool of secondary metabolites. However, accessing these compounds from their natural source proves to be an uphill battle, starting with ecological

stresses and biochemical constraints. These have led scientists to seek alternative means of accessing secondary metabolites through biotechnological processes that will increase their production in a controlled and sustainable way.

### **Natural Production of Secondary Metabolites: Challenges**

- A. Low Yield and Tissue-Specific Accumulation:** The vast majority of medicinal plants only make secondary metabolites in tiny amounts, frequently restricted to specific tissues or stages of development. For example, artemisinin—a powerful antimalarial—is synthesised in minute quantities in the glandular trichomes of *Artemisia annua*, with the yields usually 0.01%–0.8% dry weight of the plant (Tang et al., 2014). Similarly, Taxol (paclitaxel), an anticancer agent derived from *Taxus brevifolia*, is present in extremely low concentrations in the bark, rendering extraction inefficient and unsustainable (Verpoorte et al., 2000).
- B. Long Growth Cycles and Slow Regeneration:** Several high-value medicinal plants have long growth cycles and low regenerative abilities. For instance, *Taxus* species require several years to mature and produce very little Taxol, a bottleneck in drug production (Ramachandra Rao & Ravishankar, 2002).
- C. Environmental Influence:** Environmental factors, including light, temperature, soil quality, and biotic stress, predominantly influence the accumulation of secondary metabolites. This will result in significant seasonal and geographical fluctuations in the content of metabolites, potentially leading to inconsistent efficacy and quality of the drug (Verpoorte et al., 2000). Standardisation is a primary requirement in the herbal pharmaceutical and medicinal industries, and this complicates the process.
- D. Overharvesting and Biodiversity Loss:** Increased demand for phytopharmaceuticals has resulted in unsustainable exploitation of wild medicinal plants. Overexploitation, particularly of rare and slow-growing species, disrupts ecological balance and results in loss of biodiversity. *Nardostachys jatamansi*, a Himalayan plant with highly valued roots, has been listed as endangered due to over-harvesting (Ghosh, 2016).
- E. Legal and Ethical Restraints:** The medicinal plant trade globally is presently becoming more regulated. Export and collection restrictions on threatened species make raw plant material less available for drug use. Benefit-sharing and intellectual property rights issues further complicate the commercial exploitation of some plant species (Ravishankar & Chattopadhyay, 2020).

### **The Need for Biotechnological Approaches**

In light of these constraints, plant biotechnology presents potential alternatives for the improved, stable, and sustainable production of secondary metabolites.

Various new methods can circumvent the constraints of natural plant growth and extraction.

- A. Scalable and Controlled Production:** In vitro culture systems, such as callus, cell suspensions, organ cultures, and hairy root cultures, offer a well-controlled environment for the biosynthesis of secondary metabolites. Seasonal or geographical conditions do not significantly influence these systems, which are scalable for industrial-scale production using bioreactors (Ramachandra Rao & Ravishankar, 2002).
- B. Stimulation through Elicitors and Precursors:** The application of elicitor substances that trigger the plant's defence system has worked effectively in augmenting secondary metabolite production. Both abiotic (e.g., salicylic acid, UV light) and biotic (e.g., yeast cells, fungal extracts) elicitors can be used to increase biosynthesis of metabolites (Zhao et al., 2019). Precursor feeding also serves to enhance the levels of metabolic intermediates needed for specific pathways.
- C. Genetic and Metabolic Engineering:** Modern methods, such as Agrobacterium-mediated transformation, CRISPR/Cas gene editing, and RNA interference (RNAi), enable the accurate manipulation of biosynthetic genes and regulatory factors. These methods have been applied to overexpress rate-limiting enzymes and repress competing pathways, leading to higher yields of target metabolites (Zhao et al., 2019).
- D. Conservation and Sustainability:** Biotechnological approaches minimise dependence on wild plant collection and, thereby, conserve biodiversity. Hairy root and cell cultures provide a sustainable method to have bioactive compounds produced while maintaining plant populations in nature (Ravishankar & Chattopadhyay, 2020).
- E. Improved Quality and Standardisation:** More control over the manufacturing parameters is possible with in vitro systems, which results in consistent quality and chemical composition. This replicability is critical in pharmaceutical usage, where dosage accuracy and uniformity from batch to batch are paramount.

### **Biotechnological Methods for Secondary Metabolite Yield Enhancement**

Biotechnological interventions have become indispensable techniques in maximising the yield and uniformity of secondary metabolites from medicinal crops. These methods enable scientists to surmount environmental constraints, optimise the efficiency of the biosynthetic pathway, and minimise the dependency on wild plants. The major strategies involve plant tissue culture, elicitor treatment, hairy root culture, metabolic engineering, and genetic transformation.

## 1. Plant Tissue Culture Methods

It is a consistent and large-scale production platform for secondary metabolites. Cell suspension cultures and callus (undifferentiated tissue) can be raised from different explants and cultured in vitro for the production of precious compounds. These cultures are employed for the repetitive manufacture of metabolites such as shikonin, berberine, and ajmalicine (Ramachandra Rao & Ravishankar, 2002). Suspension cultures are most suitable for industrial-scale production in bioreactors. Organ cultures retain tissue differentiation, which may be essential for organ-specific metabolite formation. Shoot cultures are best suited for the manufacture of tropane alkaloids from *Atropa belladonna*. Root cultures (especially hairy roots) are successful for molecules such as resveratrol and ginsenosides (Ghosh, 2016). Benefits of this method is such that A controlled environment provides sterility and reproducibility, Culture conditions can be tailored for maximum metabolite accumulation, Best suited for the conservation of threatened species through in vitro propagation. Hairy roots are formed by infection with *Agrobacterium rhizogenes*, which converts part of its DNA (T-DNA) into the plant genome, giving rise to rapidly growing, genetically stable roots. Hairy root culture has several advantages such as Increased growth rates over natural root, Strong genetic and biochemical stability, Higher metabolite production without the presence of exogenous hormones (Verpoorte et al., 2000). *Withania somnifera* hairy roots yield more withanolides. Hairy root cultures of *Panax ginseng* enhance the accumulation of ginsenosides. (Patel, A., & Shah, S., 2021).

## 2. Elicitation

Elicitation is a process of exposing plant cultures to stress-inducing agents that trigger secondary metabolism. Elicitors simulate biotic or abiotic stress, leading to the activation of biosynthetic pathways. Biotic elicitors can be yeast extract, chitosan, and cell wall fragments of fungi whereas abiotic elicitors can be methyl jasmonate (MeJA), salicylic acid (SA), UV light, heavy metals. The application of Methyl jasmonate increases vinblastine production in *Catharanthus roseus* cultures (Zhao et al., 2019). Salicylic acid has been reported to enhance rosmarinic acid production in *Melissa officinalis* cell cultures. Elicitors stimulate transcription of genes participating in the phenylpropanoid, terpenoid, and alkaloid biosynthesis pathways, resulting in enhanced metabolite production.

## 3. Metabolic Engineering

Metabolic engineering targets the modulation of selected biosynthetic pathways for diverting flux towards the target metabolite.

### **Gene Overexpression and Silencing**

Rate-limiting enzymes such as PAL (phenylalanine ammonia-lyase) overexpression increases metabolite production. Competitive pathway silencing enhances precursor supply for the target compound (Zhao et al., 2019). PAL is a crucial regulatory step in the synthesis of numerous phenolic compounds since it is located at the intersection of primary and secondary metabolism. Research involving a variety of plant species has demonstrated that environmental conditions, including low nutrient levels, light (through its impact on phytochrome), and fungal infection, enhance the activity of PAL (Hahlbrock & Scheel, 1989). The start of transcription seems to be the point of control. For instance, fungal invasion increases the quantity of PAL in the plant by triggering the transcription of messenger RNA that codes for PAL. This, in turn, promotes the synthesis of phenolic chemicals. (Logemann et al., 1995). Transcriptomics, proteomics, and metabolomics guide the discovery of key regulatory genes and pathway bottlenecks. Integration with systems biology allows pathway reconstruction and simulation. Overexpression of ADS (amorpha-4,11-diene synthase) in *Artemisia annua* has enhanced artemisinin precursor yields (Tang et al., 2014).

### **Genetic Transformation**

Genetic engineering allows for direct manipulation of the plant genomes for increased production of secondary metabolites. Various methods can be opted such as *Agrobacterium*-mediated transformation, Particle bombardment (biolistics) or CRISPR/Cas9 genome editing (Gelvin, S. B. (2017)). Transgenic *Nicotiana tabacum* carrying *Taxus* genes yields taxol intermediates. CRISPR-edited *Salvia miltiorrhiza* lines were found to have enhanced tanshinone production (Zhao et al., 2019). Synthetic biology, coupled with CRISPR/Cas9 genome editing, will revolutionise metabolic engineering by facilitating exact gene expression control and pathway reconstruction. The reconstitution of complete biosynthetic pathways in microbial hosts (for instance, *Saccharomyces cerevisiae*) is an auspicious approach for compounds such as artemisinin and Taxol (Zhou et al., 2021). Genes participating in metabolite biosynthesis can be rapidly discovered owing to high-throughput sequencing technologies. The prediction of pathway bottlenecks and optimal engineering targets is facilitated through the use of computational methods, such as metabolic flux balance analysis (FBA) (Scherer et al., 2022).

### **Case Studies and Applications in Specific Medicinal Plants**

Some medicinal plants have been optimally subjected to biotechnological interventions for enhanced production of pharmaceutically important secondary metabolites. These case studies demonstrate the applied significance of tissue

culture, elicitation, hairy root technology, and metabolic engineering in actual practice.

➤ **Ashwagandha (*Withania somnifera*)**

Ashwagandha is a commonly employed medicinal herb in Ayurveda, mainly attributed to its anti-inflammatory and adaptogenic properties. Hairy root cultures produced by *Agrobacterium rhizogenes* have increased the content of withanolides, particularly withaferin A and withanolide D (Singh et al., 2020). The use of elicitors like methyl jasmonate and salicylic acid also further promoted secondary metabolite biosynthesis through the activation of important genes such as HMGR and SQS (Patel & Shah, 2021).

➤ ***Artemisia annua* (Sweet Wormwood)**

The poor natural yield of artemisinin has spurred widespread biotechnological work. Transgenic strategies have entailed overexpression of amorpha-4,11-diene synthase (ADS) and CYP71AV1, resulting in increased artemisinin precursors (Chen et al., 2021). CRISPR-Cas9 editing has been used to knock out competing pathways, driving flux towards artemisinin biosynthesis (Kumar et al., 2022).

➤ ***Panax ginseng* (Korean Ginseng)**

The therapeutic potential of ginseng is due to its immunomodulatory and neuroprotective effects. Hairy root cultures have been reported with higher biomass and ginsenoside content in comparison with regular root cultures. Overexpression of genes for squalene synthase and dammarenediol synthase increased the production of specific ginsenosides (Lee et al., 2019). Transcriptomic profiling assisted in the identification of transcription factors controlling the ginsenoside biosynthetic pathway (Cho et al., 2021).

## **Conclusion**

Our ability to increase the production of secondary metabolites in medicinal plants has been dramatically improved due to biotechnological treatments. Traditional constraints, including low yield, seasonal dependence, and slow growth rates, can now be overcome with metabolic engineering, genetic transformation, and omics-guided approaches. Targeted and effective modification of biosynthetic pathways has been made possible by the use of CRISPR/Cas9, transgenic strategies, and elicitor-based techniques. Additionally, there are encouraging opportunities for pathway optimisation and scalable manufacturing through the combination of synthetic biology and systems biology. These developments open the door to new drug discovery, in addition to facilitating the commercial and sustainable synthesis of valuable phytochemicals. The public's acceptance of genetically modified organisms, ecological effects, and governmental barriers must be addressed, nevertheless.

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