STM JOURNALS

Research & Reviews: A Journal of
Biotechnology

https://journals.stmjournals.com/rrjobt/

ISSN: 2231-3826 (Online)
ISSN: 2347-7245 (Print)
Volume 16, Issue 2, 2026
May–August
DOI (Journal): 10.37591/RRJOBT

Research RRJOBT

CRISPR-Based Enhancement of Secondary Metabolites in *Artemisia annua*: Focus on Artemisinin Biosynthesis

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Abstract

Medicinal plants are natural biofactories producing secondary metabolites with significant therapeutic value, including terpenoids, alkaloids, and phenolics. Among these, artemisinin, a sesquiterpene lactone from Artemisia annua L. is the cornerstone of global antimalarial therapy along with additional anticancer, antiviral, and antimicrobial activities. However, artemisinin content in the native plants is inherently low (typically 0.01–0.8% dry weight), restricting affordable and consistent supply. Conventional approaches to increase production include classical breeding, single-gene overexpression, and RNAi-based suppression of competing pathways. These approaches have achieved only incremental improvements and are constrained by limited precision, off-target pathway effects, and regulatory challenges as they include foreign DNA introduction. CRISPR/Cas9-based genome editing has emerged as a transformative solution, enabling precise and multiplexed modification of endogenous genes governing secondary metabolic pathways. In A. annua, CRISPR strategies targeting the squalene synthase (SQS) gene which diverts the shared farnesyl diphosphate precursor toward competing sterol biosynthesis are expected to substantially increase artemisinin flux. Disruption of negative transcriptional regulators (including AaMYB15, AabHLH2/3, AabHLH5, and TrichomeLess Regulator 3) and activation of positive regulators (AaWRKY9, AaMYC3) by CRISPR provide strategies to enhance pathway gene expression. Integration of CRISPR with single-nucleus transcriptomics, comprehensive multi-omics databases (ArtemisiaDB), and AI-assisted sgRNA design platforms maximizes the precision, efficiency, and safety of editing operations. The combined effect of pathway flux redirection, negative regulator knockout, and trichome engineering is expected to achieve artemisinin yields significantly above those of current high-producing varieties. These advances hold the potential to reduce production costs, stabilize global supply, and improve access to life-saving antimalarial medicines. The principles developed in A. annua are broadly transferable to other medicinal plant systems, positioning CRISPR-based metabolic engineering as a cornerstone of next-generation plant biotechnology for sustainable pharmaceutical production.

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Received Date: June 25, 2026
Accepted Date: July 20, 2026
Published Date: August 01, 2026

Citation: Nivedita Mishra, Rakshita Shrivastava, Deeksha Mital, Palak Bhatia. CRISPR-Based Enhancement of Secondary Metabolites in *Artemisia annua:* Focus on Artemisinin Biosynthesis. Research & Reviews: A Journal of Biotechnology. 2026; 16(2): 23–35p.

Keywords: Artemisinin, CRISPR/Cas9, secondary metabolites, *Artemisia annua*, genome editing, glandular secretory trichomes

INTRODUCTION

Plants have been used as natural sources of therapeutic compounds throughout human history. They continue to serve as irreplaceable biofactories producing a vast diversity of secondary metabolites used for various therapeutic purposes. *Artemisia annua* L., a traditional Chinese medicinal herb with potent antimalarial value has gained global prominence for production of artemisinin, a sesquiterpene. The discovery of artemisinin in 1972 represented one of the most significant advances in tropical medicine of the

twentieth century. The artemisinin is a secondary metabolite involved in allelopathic and defense pathway of plant [1].

Since Nobel Prize in Physiology or Medicine in 2015 was awarded the for this discovery, the therapeutic potential of *Artemisia annua* L., gained significant attention from researchers worldwide [2]. Beyond malaria, artemisinin and its derivatives have shown many other pharmacological properties including anti-cancer, antiviral, and anti-inflammatory activities [3]. Moreover, *A. annua* extracts have been studied for modulation of YAP signaling pathways affecting cognitive deficits in Alzheimer's disease also [4].

World Health Organization (WHO) now recommend Artemisinin-based combination therapies (ACTs) for uncomplicated malaria caused by *Plasmodium falciparum* thus increasing global demand for artemisinin.

The artemisinin yield differs between cultivars however, the natural content of artemisinin (0.01–0.8% of dry weight) in *A. annua* is extremely low. Thus, demand supply constrains persistent bottleneck for large-scale, affordable production [2, 5, 6]. The current production methods for artemisinin rely predominantly on agricultural extraction and semi-synthetic production methods using engineered yeast. These approaches have significant limitations in cost, scalability, and supply chain stability [7, 8].

Recent studies of the genomic architecture of *A. annua* for artemisinin production revealed that various endogenous regulatory genes and some negative regulators affect its production and variable copy numbers across these genes correlate with differences in artemisinin yield between cultivars [1, 6]. The development of the ArtemisiaDB, a high-resolution multi-omics expression atlas, represents a major recent advance in providing the genetic roadmap required for engineer artemisinin production in plant [9]. By integrating CRISPR/Cas9 with such new and emerging technologies, it is possible to develop next-generation medicinal plant crops with greatly enhanced bioactive content aiding in the sustainable production of valuable natural compounds like artemisinin.

This review addresses how CRISPR-based genome editing is poised to resolve the production bottleneck for artemisinin. We discuss the importance of secondary metabolites, limitations of conventional production approaches, the mechanistic basis of CRISPR technology, current knowledge of the CRISPR-based metabolic engineering of the artemisinin pathway, and the integration of CRISPR with advanced omics and synthetic biology platforms.

IMPORTANCE OF SECONDARY METABOLITES

Secondary metabolites are low-molecular-weight organic compounds produced by plants as non-essential constituents for primary growth and development, but they play critical supporting roles in plants and are pharmacologically important phytochemicals. They serve as chemical defenses against pathogens, herbivores, UV radiation, and competing plant species. For human, these phytochemicals constitute the molecular basis of a large proportion of clinically approved drugs.

Secondary metabolites are broadly classified into three major groups: alkaloids such as morphine, vincristine, and berberine, etc., terpenoids such as artemisinin, taxol, and vinblastine, etc., and phenolics including flavonoids, tannins, and phenolic acids. Alkaloids are widely used in pain management, oncology, and antimicrobial therapy; Terpenoids are potent anticancer and antimalarial compounds and Phenolics, are antioxidant, anti-inflammatory, and cardioprotective phototherapeutic compounds.

The biosynthesis of these compounds is tightly regulated by genetic, developmental, and environmental factors. In *A. annua*, artemisinin accumulates in peltate glandular secretory trichomes

(GSTs). These GSTs are specialized epidermal structures that function as metabolic factories and work for synthesizing, storing, and secreting the secondary metabolic compounds including artemisinin [10]. Artemisinin content in these structures rarely exceeds 1% of the plant's dry weight hence, making affordable large-scale extraction challenging and newer biotechnological tools are required to enhance its production and accumulation [3].

The low and variable yields of secondary metabolites – such as artemisinin – are due to many intrinsic biosynthetic constraints as well as also due to the highly variable influence of outside factors such as abiotic stresses, light exposure, and soil conditions, etc. [11]. Environmental stress, including UV light and drought, can significantly enhance artemisinin biosynthesis, suggesting that pathway activity is responsive to external stimuli and may be leveraged through both agrotechnical and molecular approaches [8, 12, 13].

CONVENTIONAL TECHNIQUES TO INCREASE PRODUCTION AND THEIR LIMITATIONS

Historically, attempts to increase artemisinin yield have involved agronomic approaches such as cultivar selection and optimized cultivation practices, genetic improvement through classical plant breeding, etc. Classical breeding has produced high-yielding *A. annua* hybrids, with some elite varieties achieving artemisinin contents 1.0–1.4% in flower tissues at peak bloom. However, conventional breeding is time-consuming, limited by the narrow natural genetic variation within the species, and often yields inconsistent results across different agro-climatic zones [8].

Transgenic approaches involving the overexpression of structural genes – such as ADS (amorpha-4,11-diene synthase), CYP71AV1, and DBR2 within the artemisinin biosynthetic pathway – have achieved moderate improvements. Qamar et al. (2024) reported that co-overexpression of six key biosynthetic enzymes in *A. annua* led to a significant increase in artemisinin production. However, these gains were limited by competing pathways and by regulatory constraints at the transcriptional level imposing production limitations [14].

The review by Wani et al. (2021) provides a comprehensive survey of alternative, classical, and transgenic approaches to enhancing artemisinin content and delivery from *A. annua*. It highlights that while each approach has yielded incremental gains, none has provided the combination of precision, efficiency, and regulatory acceptability required for large-scale crop improvement. Key limitations include (i) the inability to specifically suppress competing metabolic flows without collateral disruption of other pathways, (ii) the epigenetic silencing of transgenes over successive plant generations, (iii) regulatory hurdles associated with GMO crops, and (iv) the complexity of achieving coordinated expression of multiple pathway genes [8].

A systematic review by Zeinali et al 2025 explained alternative artemisinin production strategies encompassing in vitro cultures, semi-synthesis in engineered yeast, and hairy root systems and highlighted that none of these approaches currently surpass *in planta* production at the commercial scale, reinforcing the continuing importance of improving artemisinin yield in the native plant [15]. Thus, a more targeted, precise, and efficient molecular tool is required that can selectively modify the endogenous genome of *A. annua* without the introduction of foreign DNA. This will reduce the regulatory complications of traditional GMO frameworks and will also increase public acceptance.

WHY CRISPR?

The CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) system has emerged as the pre-eminent tool for precise genome editing. First described in its molecular detail by Jinek et al. (2012) and subsequently developed for genome editing in eukaryotes, the CRISPR/Cas9 system exploits a two-component mechanism: a Cas9 endonuclease that generates a double-strand break (DSB) in target DNA, and a single guide RNA (sgRNA) that

directs Cas9 to the specific genomic location adjacent to a PAM (protospacer adjacent motif) sequence [16]. CRISPR/Cas9 allows multiple genes to be targeted simultaneously through a process of multiplexed editing. In this process competitive branches and regulatory nodes are modified simultaneously giving manyfold impact. This feature is of particular relevance in secondary metabolite pathway engineering, where many pathways are linked impacting outcome hence in tandem pathway modification is more effective [16, 17].

In the context of medicinal plant biotechnology, CRISPR offers several decisive advantages over conventional transgenic approaches. CRISPR linked modifications have better editing efficiency, ease of design, and low cost compared to prior technologies thus supporting functional genomics research in medicinal plants. It achieves targeted disruption of endogenous genes (gene knockout), precise insertion of beneficial sequences at defined loci (knock-in), or modulation of gene expression through catalytically inactive dCas9 fused to transcriptional activators or repressors (CRISPRa and CRISPRi), without the mandatory introduction of foreign DNA. Thus, CRISPR-edited plants in which no foreign sequence remains in the final edited line do not come under GMO regulations, making it easier for manufacturers to go for commercial production and also better acceptance in public domain.

Kim et al. (2016) demonstrated the utility of CRISPRi for balancing a biosynthetic mevalonate pathway in *Escherichia coli*, achieving a marked increase in terpenoid production which can be extended to plant systems. Das et al. (2024) provided a comprehensive review of the application of CRISPR/Cas-based gene editing for the enhancement of specialized metabolites in medicinal plants, documenting cases across alkaloid, terpenoid, and phenolic pathways. The review highlighted that CRISPR-mediated modulation of both structural genes and transcription factors can produce substantial and stable increases in target metabolite accumulation, and discussed how advanced Cas variants (e.g., Cas12a, base editors, prime editors) are used for improving the precision and scope of editing applications [18, 19].

TYPES OF SECONDARY METABOLITES AND THEIR RELEVANCE TO CRISPR

The application of CRISPR technology to secondary metabolite production can be used for any of the three major classes of plant specialized metabolites: alkaloids, terpenoids, and phenolics. Each class presents specific targets and strategies for CRISPR-based enhancement.

Alkaloids

Alkaloids are nitrogen-containing compounds derived primarily from amino acid precursors. Representative medicinal alkaloids include morphine and codeine (from *Papaver somniferum*), vincristine and vinblastine (from *Catharanthus roseus*), and berberine (from *Coptis japonica*). CRISPR-based approaches have been explored for redirecting alkaloid biosynthetic flux. The disruption of the competitive branch point enzyme CYP80B1 by CRISPR in *P. somniferum* has been shown to modulate benzylisoquinoline alkaloid profiles, demonstrating the feasibility of editing this pathway to shift the balance between morphine, codeine, and other opioids [19].

Terpenoids

Terpenoids constitute the largest and most structurally diverse class of plant secondary metabolites. Among these, sesquiterpene lactones artemisinin is of great therapeutic importance. Terpenoid biosynthesis follows two parallel routes: the methylerythritol phosphate (MEP) pathway in plastids and the mevalonate (MVA) pathway in the cytosol/endoplasmic reticulum, both contributing precursors to the sesquiterpene-specific farnesyl diphosphate (FPP) intermediate. CRISPR editing of genes within and flanking these pathways particularly those encoding competing enzymes – such as squalene synthase (SQS) – is a primary strategy for increasing flux toward artemisinin [20].

Guo et al. (2025) conducted a genome-wide analysis of oxidosqualene cyclase (OSC) genes in *A. annua* and demonstrated that individual OSC members of this gene family exhibit distinct expression

patterns across tissues and developmental stages. The identification of OSC genes that compete with the artemisinin pathway for FPP precursors provides additional CRISPR targets beyond SQS [21].

Phenolics

Phenolic compounds encompassing flavonoids, hydroxycinnamic acids, stilbenes, and tannins share the common phenylpropanoid pathway as their biosynthetic origin. In *A. annua*, flavonoids (including artemetin and casticin) co-accumulate with artemisinin in GSTs and have been shown to synergistically enhance antimalarial activity. CRISPR editing of key phenylpropanoid pathway enzymes, such as chalcone synthase or flavanone 3-hydroxylase, allows rebalancing of carbon flux between phenolics and terpenoids, providing an additional dimension of metabolic engineering strategy in this plant [19].

The metabolic engineering platform thus requires careful systems-level understanding of how modification of one pathway influences flux through others. The emergence of comprehensive multi-omics databases – such as ArtemisiaDB – greatly facilitates this systems-level analysis by providing integrated transcriptomic, metabolomic, and genomic information across the entire *A. annua* biosynthetic network [9].

CRISPR-BASED METABOLIC PATHWAY ENGINEERING

The strategy of CRISPR-based metabolic pathway engineering in *A. annua* is grounded in a detailed molecular map of the artemisinin biosynthetic pathway, which has been fully elucidated from FPP to artemisinin. The pathway involves the sequential action of ADS (amorpha-4,11-diene synthase), CYP71AV1 (a multifunctional P450), DBR2 (double bond reductase 2), and ALDH1 (aldehyde dehydrogenase), culminating in the non-enzymatic photochemical conversion of dihydroartemisinic acid to artemisinin (Figure 1) [22].

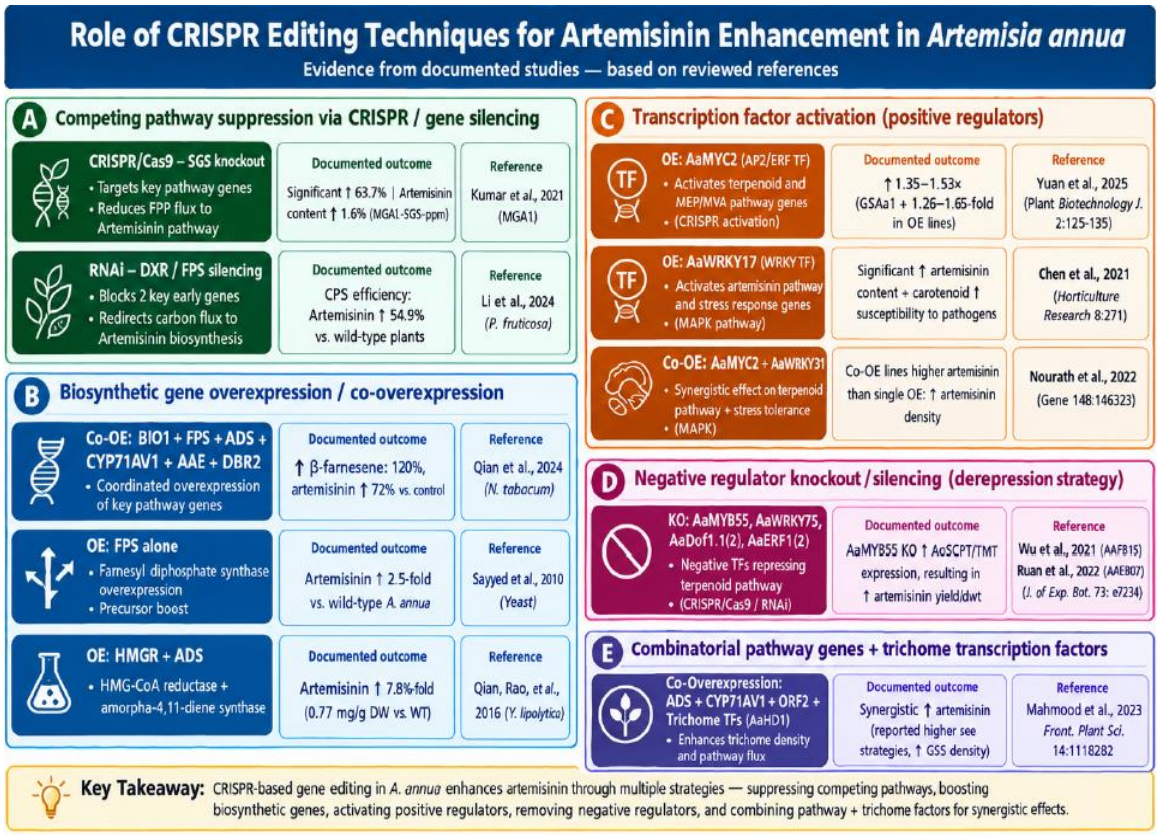

Figure 1. Role of CRISPR and gene-editing techniques for Artemisin production enhancement in *Artemisia annua* L.

Note: OE – Overexpression, KO – Knockout, TF – Transcription Factor, GST – Glandular Secretory Trichome.

Disruption of Competing Pathways

A critical CRISPR strategy involves the targeted disruption of metabolic branches that compete with artemisinin biosynthesis for the shared FPP precursor. The squalene synthase (SQS) gene, which diverts FPP into sterol biosynthesis, is the most studied target. Koerniati and Simanjuntak (2020) proposed a CRISPR/Cas9-based strategy to disrupt SQS in *A. annua*, with computational design of crRNA targeting the SQS coding sequence, predicting that this knockout would redirect FPP flux away from sterols and toward artemisinin production. Both artemisinin and sterols share FPP as a common precursor; thus, suppressing SQS activity increases the substrate pool available for ADS and the downstream artemisinin biosynthetic enzymes [20]. This in silico approach was subsequently extended through the design of crRNA sequences targeting the SQS locus in *A. annua* using bioinformatics tools, confirming the feasibility of CRISPR-mediated disruption of the sterol competing pathway [20]. Though, most of the existing literature has concentrated on finding potential target genes, computationally designing CRISPR guides, or studying gene functions, this is among very few studies that have shown successful gene modification using CRISPR technology to increase the production of artemisinin in A. annua and showing evidence of metabolic improvement [19, 20].

Transcription Factor Editing

In addition to structural gene editing, the transcriptional regulatory network governing artemisinin biosynthesis presents rich targets for CRISPR intervention. Over 60 transcription factors (TFs) from 14 families have been identified as regulators of artemisinin pathway genes. These include WRKY, MYB, bHLH, bZIP, MADS-box, AP2/ERF, and SHI family members [22–24].

Among the positive regulators, AaWRKY9 has been shown by Fu et al. (2021) to directly bind the promoters of AaDBR2 and AaGSW1, thereby promoting artemisinin accumulation in a light- and jasmonate (JA)-dependent manner. The JA-regulated complex involving AaGSW1, AaYABBY5, and AaWRKY9, characterized by Kayani et al. (2023), further demonstrates the multilayered transcriptional architecture that CRISPR can be used to fine-tune [13, 22, 24].

Negative regulators are equally attractive CRISPR targets, as their disruption may release pathway genes from transcriptional repression. AaMYB15, characterized by Wu et al. (2021) as an R2R3-MYB transcription factor that negatively regulates artemisinin biosynthesis, is a prime candidate for CRISPR knockout, with predicted release of pathway genes from suppression upon its disruption [25]. Similarly, AabHLH2 and AabHLH3 function antagonistically with the positive regulator AaMYC2 in artemisinin biosynthesis regulation. As demonstrated by Shen et al. (2022); their targeted deletion by CRISPR would be predicted to increase artemisinin pathway expression [26].

AabHLH5, identified by Xu et al. (2025) as a transcription factor that participates in JA signaling and negatively regulates artemisinin biosynthesis, provides another recently validated CRISPR target. The chromatin accessibility landscape of the artemisinin pathway, analyzed by Zhou et al. (2021), further revealed that chromatin remodeling at key pathway gene loci is closely associated with biosynthetic regulation, suggesting that CRISPR-based epigenome editing tools could modulate chromatin accessibility to increase transcriptional output [11, 27].

Trichome Development Engineering

Since artemisinin biosynthesis is exclusively localized to GSTs, increasing GST density on the plant surface is a major engineering goal. CRISPR-mediated disruption of negative regulators of trichome development has been shown to substantially increase trichome number. The gene TrichomeLess Regulator 3 (TLR3), characterized by Dong et al. (2024), was shown to negatively regulate trichome initiation and cuticle biosynthesis in *A. annua*; its CRISPR-mediated knockout would be expected to increase trichome density and consequently artemisinin production [28].

In parallel, the positive regulator AaMYC3, identified by Yuan et al. (2025) as a bridge between GST density regulation and artemisinin biosynthesis in a JA-dependent manner, represents a target for

CRISPR transcriptional activation (CRISPRa), potentially increasing both trichome number and pathway gene expression simultaneously [29].

Hassani et al. (2023) demonstrated that co-transformation of artemisinin biosynthetic pathway genes with trichome-specific transcription factors resulted in an elevation of artemisinin content in *A. annua*, validating the principle that combined targeting of pathway structural genes and trichome developmental regulators is an effective strategy [17].

Promoter Engineering

Promoter and transcriptional regulator editing offers a complementary CRISPR strategy. Li et al. (2022) identified a truncated AaActin1 promoter as a candidate tool for driving strong expression of artemisinin biosynthetic genes in *A. annua*, demonstrating that promoter engineering can be used to enhance transcriptional output [30]. CRISPR-mediated promoter modifications – such as deletion of repressor binding sites or introduction of activator motifs – could further increase the transcriptional capacity of key pathway genes without altering coding sequences [30].

CRISPR EDITING IN *ARTEMISIA ANNUA*

The practical application of CRISPR/Cas9 to *Artemisia annua* follows a defined experimental workflow that integrates molecular biology, plant cell biology, and analytical chemistry. The detail described here explain the major steps and relevant scientific evidence for each stage.

Target Gene Identification and sgRNA Design

Target gene identification in *A. annua* is now greatly facilitated by the availability of high-quality chromosome-level genome assemblies and comprehensive transcriptomic resources. The allele-aware genome assembly details given by Xu et al. (2022) provide the reference sequence framework within which coding sequences, regulatory regions, and variant loci that can be precisely mapped [27]. ArtemisiaDB further provides tissue-resolved expression data, enabling the identification of genes that are both highly expressed in GSTs and functionally relevant to artemisinin biosynthesis [9].

sgRNA design tools including CRISPR-PLANT, Cas-OFFinder, and deep-learning-based platforms are used to identify highly specific 20-nucleotide protospacer sequences with minimal off-target potential. Bao and Liu (2024) reported the development of DeepFM-Crispr, a deep learning model for the prediction of CRISPR on-target effects, providing improved confidence in sgRNA selection for medicinal plant applications [31].

Vector Construction and Transformation

CRISPR constructs for plant transformation typically include the SpCas9 coding sequence (or a codon-optimized variant), one or more sgRNA expression cassettes driven by plant U6 or U3 promoters, a selectable marker (commonly kanamycin or hygromycin resistance), and appropriate plant regulatory elements. These components are assembled into a binary T-DNA vector suitable for *Agrobacterium tumefaciens*-mediated transformation.

In *A. annua*, *Agrobacterium*-mediated transformation has been the predominant delivery method. Li et al. (2022) described an optimized pipeline for increasing artemisinin production through the simultaneous promotion of GST formation and reconstruction of the artemisinin biosynthetic pathway in *A. annua*, using *Agrobacterium*-mediated co-expression of multiple transgenes [22, 30].

Target Editing for SQS Silencing

Among the CRISPR targets identified for modification in A. annua, the greatest amount of effort has been invested into modifying squalene synthase (SQS). It makes sense from the perspective of metabolism, since SQS acts on the substrate, farnesyl pyrophosphate (FPP), directing it towards the synthesis of steroids in the competition with the synthesis of artemisinin [25–32].

The hypothesis is that knockout of SQS through CRISPR/Cas9 will increase the amount of FPP that will be used for the artemisinin biosynthesis. But it should be mentioned that there is no experiment demonstrating that knockout of SQS by CRISPR/Cas9 results in an increase in artemisinin production in *Artemisia annua*. So far, all the publications are based either on computational analysis, or on target recognition, or on theoretical considerations [8, 19, 20, 33].

That is what motivated Koerniati and Simanjuntak (2020) to construct CRISPR/Cas9 constructs aimed at disruption of SQS gene, relying on the previous RNAi studies in similar species, where knockdown of SQS led to higher content of sesquiterpenoids [20]. The mechanism of action is as follows: Cas9 induces double strand breaks followed by NHEJ repair with insertion/deletion of nucleotides and consequently, disruption of SQS open reading frame. This is supposed to lead to the decrease of FPP flow into sterols and its redirection into amorpha-4,11-diene – the initial molecule of artemisinin biosynthesis. But this measure may not be enough, since Tang et al. (2023) reviewed all metabolic engineering approaches in A. annua and concluded that combination of SQS knockdown with overexpression of pathway positive regulators resulted in better effect [23].

Despite the successful application of the CRISPR/Cas9 system to edit the Squalene Synthase (SQS) gene in *Artemisia annua*, most of the experiments conducted on the genome editing in this species have mainly focused on verifying the edits through PCR and sequencing techniques [34, 35]. On the other hand, the quantification of the amount of artemisinin accumulated in the edited *Artemisia annua* plants has not been done much in the literature. This shows that there is a need for validating CRISPR knockout lines which accumulate higher amounts of artemisinin [34, 36].

Selection, Validation, and Metabolite Quantification

Transformed plant cells are cultured on selective media supplemented with kanamycin or hygromycin to identify stably transformed lines. The edited plants are then screened by PCR amplification of the target locus, followed by Sanger sequencing or next-generation sequencing to confirm the presence of insertions/deletions at the sgRNA target site. For CRISPR applications aimed at gene knockout, successful editing is confirmed by the identification of frameshift mutations or premature stop codons within the coding sequence.

Artemisinin content in edited lines is quantified using high-performance liquid chromatography (HPLC) with UV detection, or by LC-MS/MS for higher sensitivity and specificity. Advanced metabolic engineering strategies for *A. annua*, as reviewed by Li et al. (2024), have incorporated metabolomics-based profiling of entire terpenoid and flavonoid profiles alongside targeted artemisinin quantification to assess the broader metabolic consequences of pathway manipulations [6, 22].

INTEGRATION WITH ADVANCED TECHNOLOGIES

The application of CRISPR to artemisinin metabolic engineering operates by its integration with advanced omics platforms, synthetic biology, artificial intelligence, and plant tissue culture systems.

Genomics and Transcriptomics

Comprehensive genomic and transcriptomic resources are now available for *A. annua* and provide the foundation for rational CRISPR target selection. The allele-aware chromosome-level genome assembly reported by Liao et al. (2022) provides a high-resolution reference for identifying gene structures, regulatory elements, and genomic variants associated with artemisinin yield differences between cultivars. ArtemisiaDB, an integrated multi-omics database, further provides Gene Ontology annotations, KEGG pathway mappings, and tissue-specific expression profiles that enable the prioritization of CRISPR targets based on expression patterns across GSTs, leaves, and other tissues [6, 9].

The single-nucleus transcriptomic atlas of GST development generated by Zhang et al. (2025) reveals not only the cell-type specificity of artemisinin biosynthesis but also the gene regulatory

networks active at each developmental stage, enabling CRISPR interventions to be designed to act at specific times or developmental stage of *A. annua* [32].

Metabolomics

Metabolomics provides a quantitative record of the metabolic consequences of genetic manipulations, enabling researchers to assess whether CRISPR-edited lines show the expected increase in target metabolites and to detect unintended metabolic distresses in other pathways. Kim et al (2016) demonstrated that environmental stresses modulate the metabolome of *A. annua* by activating artemisinin biosynthesis genes, highlighting the importance of comprehensive metabolite profiling in assessing engineering outcomes [18].

Synthetic Biology

Synthetic biology approaches complement CRISPR by providing additional tools for pathway reconstruction and optimization. These include the use of synthetic promoters, gene stacking strategies, and metabolic flux balance analysis to predict optimal configurations of gene expression. Tang et al. (2023) reviewed the integration of synthetic biology with metabolic engineering of artemisinin biosynthesis, noting that the reconstruction of entire pathway modules with codon-optimized sequences and trichome-specific promoters can substantially enhance metabolic output [23].

Zaman and Park (2026) reviewed recent advances in functional genomics and plant biotechnology for enhancing the therapeutic potential of medicinal plants, placing CRISPR within a broader innovation framework that includes synthetic biology, artificial intelligence-assisted target prediction, and scalable bioproduction systems. Their integrated framework explicitly links genomic discovery with sustainable healthcare application, underscoring the translational potential of CRISPR-based metabolic engineering [37].

Artificial Intelligence and Deep Learning

The integration of artificial intelligence (AI) and machine learning with functional genomics is accelerating the identification of high-confidence CRISPR targets and the prediction of editing outcomes in medicinal plants. AI-assisted platforms for sgRNA efficiency prediction – such as DeepFM-Crispr (Bao and Liu, 2024) – provide improved accuracy in target site selection, reducing off-target effects and increasing the proportion of successfully edited plants in transformation experiments. Furthermore, AI-based pathway modelling can predict the metabolic consequences of combinatorial gene editing, enabling researchers to design multi-target CRISPR strategies with greater confidence [31].

Plant Tissue Culture

Plant tissue culture remains an indispensable enabling technology for the selection and propagation of CRISPR-edited *A. annua* lines. Transformed cells are regenerated into complete plants through somatic embryogenesis or organogenesis on hormone-supplemented media, with subsequent rooting and acclimatization. The optimization of tissue culture protocols for *A. annua* particularly transformation efficiency and plant regeneration rates directly impacts the throughput of CRISPR editing experiments.

Al-Khayri et al. (2022) provided a comprehensive review of biotechnological approaches for artemisinin production from *A. annua*, including tissue culture platforms such as hairy root cultures, cell suspension cultures, and shoot apex cultures. These systems can be combined with CRISPR editing to produce edited in vitro systems that may serve as production platforms or as rapid screening tools for evaluating the metabolic impact of specific genetic modifications [38].

Terpenoid Engineering Integration

The recent review by Guo et al. (2025) on metabolic engineering of terpenoid biosynthesis in medicinal plants from genomic insights to biotechnological applications provides an authoritative

synthesis of how CRISPR fits within the broader landscape of terpenoid pathway engineering. The authors highlight that the combination of high-quality reference genomes, single-cell transcriptomics, and CRISPR gene editing is now enabling the rapid functional characterization of candidate pathway genes and regulators, significantly accelerating the iterative cycle of discovery and engineering [21].

OUTCOMES AND IMPACT

The convergence of CRISPR genome editing with the molecular biology of artemisinin biosynthesis in *A. annua* is generating tangible outcomes with far-reaching implications for global healthcare, pharmaceutical production, and plant biotechnology.

Enhanced Artemisinin Production

The primary anticipated outcome of CRISPR-based metabolic engineering in *A. annua* is a substantial increase in artemisinin content, beyond what has been achieved through conventional breeding or single-gene transgenic approaches. The CRISPR-mediated disruption of SQS pathways, as proposed by Koerniati and Simanjuntak (2020), combined with the disruption of negative transcriptional regulators, such as AaMYB15, TLR3, and AabHLH5, and the activation of positive regulators such as AaWRKY9 and AaMYC3, is expected to produce synergistic increases in artemisinin accumulation [13, 20, 25, 27–29].

The review by Tang et al. (2023) and Li et al. (2024) demonstrated that multi-target metabolic engineering strategies combining pathway flux enhancement, competing branch suppression, trichome density increase, and transcription factor modulation, etc., consistently achieve greater yield improvements than single-target approaches [21, 22]. CRISPR is uniquely positioned to implement such multi-target strategies efficiently, particularly through multiplexed editing using multiple sgRNAs delivered simultaneously.

Reduced Production Costs and Improved Supply

Currently, the global supply of artemisinin remains concentrated in a small number of agricultural producer nations, with China and Vietnam accounting for over 80% of production [2]. This concentration creates supply chain vulnerability and price volatility that adversely affects access to ACTs in malaria-endemic regions. CRISPR-enhanced *A. annua* varieties with significantly higher artemisinin yields would reduce the land area required for production, lower extraction costs per unit of artemisinin, and enable geographically distributed cultivation particularly in sub-Saharan Africa, where the disease burden is highest [5].

The review by Lee et al. (2023) discussed the potential for artemisinin production in South Africa using optimized agro-photovoltaic systems, noting that *A. annua* plants grown under partial solar panel shading produced approximately 20% more artemisinin than controls. When combined with CRISPR-enhanced high-artemisinin cultivars, such integrated production systems could substantially improve artemisinin availability in the sub-Saharan region [2].

Consistency and Reduction of Environmental Variability

A persistent challenge with plant-derived pharmaceuticals is the variability in secondary metabolite content driven by environmental fluctuations. By embedding enhanced metabolic flux at the genetic level through stable CRISPR edits, high-artemisinin plants maintain elevated production across variable environmental conditions. This genetic stabilization of metabolite yield is a crucial improvement over approaches that rely on optimized growth conditions that may not be replicable across diverse agricultural settings.

Broader Application to Other Medicinal Plants

The principles and technologies developed for CRISPR-based artemisinin engineering in *A. annua* are directly applicable to other high-value medicinal plant systems. Nath (2025) reviewed

biotechnological enhancement of secondary metabolites across a range of medicinal plants, emphasizing the generalizable nature of the CRISPR toolbox [39]. Gao et al (2025) further highlighted targeted biotechnological interventions for high-value secondary metabolite production, documenting successful CRISPR applications in the engineering of alkaloids, phenolics, and other phytopharmaceuticals [33]. These reviews underscore the growing pharmaceutical relevance of CRISPR-edited medicinal plant systems, particularly for the production of compounds used in oncology [33, 39].

Challenges and Future Perspectives

Despite its considerable promise, the application of CRISPR to *A. annua* and other medicinal plants faces several challenges. The transformation efficiency of *A. annua* via Agrobacterium-mediated approaches remains lower than in model systems, and the regeneration of fertile, stably edited plants from transformed callus is technically demanding. Off-target editing although substantially reduced with improved sgRNA design and high-fidelity Cas9 variants, still it remains a concern in the context of regulatory approval and biosafety assessment.

Future advances are anticipated in several areas. The deployment of base editors and prime editors which achieve precise single-nucleotide substitutions or small insertions/deletions without generating double-strand breaks will further expand the precision and safety profile of CRISPR interventions in medicinal plants. The integration of CRISPR with high-throughput phenotyping and AI-driven metabolic modelling, as advocated by Zaman and Park (2026), will accelerate the identification of optimal multi-target editing strategies. Furthermore, the development of non-integrating CRISPR delivery systems – such as virus-induced gene silencing (VIGS)-based CRISPR or ribonucleoprotein (RNP) delivery – will enable transient editing without stable transformation, potentially bypassing some regulatory barriers [37].

CONCLUSION

To conclude, the biotechnological enhancement of secondary metabolites represents one of the most significant near-term opportunities in plant sciences, with direct implications for global health, pharmaceutical affordability, and sustainable agriculture. CRISPR, as the most precise and efficient tool currently available for plant genome editing, is at the forefront of this transformation.

Acknowledgment

The authors are grateful to the Department of Biotechnology, Jaypee Institute of Information Technology, Noida, Uttar Pradesh, India, for providing the necessary facilities to execute this work.

REFERENCES

1. Yin Q, Xiang L, Han X, Zhang Y, Lyu R, Yuan L, et al. The evolutionary advantage of artemisinin production by Artemisia annua. Trends Plant Sci. 2025;30(2):213–26.
2. Lee BJ, Weyers M, Haynes RK, van der Kooy F. Discovery of artemisinin in Artemisia annua, its current production, and relevance to sub-Saharan Africa. S Afr J Bot. 2023;153:21–7.
3. Wei ZX, Pan JP, Li Y. Artemisinin G: A sesquiterpene from Artemisia annua. Planta Med. 1992;58(3):300.
4. Zhou W, Lei B, Yang C, Silva M, Xing X, Yu H, et al. Artemisia annua extract improves the cognitive deficits and reverses the pathological changes of Alzheimer's disease via regulating YAP signaling. Int J Mol Sci. 2023;24(6):5259.
5. Ikram NK, Simonsen HT. A review of biotechnological artemisinin production in plants. Front Plant Sci. 2017;8:1966.
6. Liao B, Shen X, Xiang L, Guo S, Chen S, Meng Y, et al. Allele-aware chromosome-level genome assembly of Artemisia annua reveals the correlation between ADS expansion and artemisinin yield. Mol Plant. 2022;15(8):1310–28.
7. Xu J, Liao B, Yuan L, Shen X, Liao X, Wang J, et al. 50th anniversary of artemisinin: From the discovery to allele-aware genome assembly of Artemisia annua. Mol Plant. 2022;15(8):1243–6.

8. Wani KI, Choudhary S, Zehra A, Naeem M, Weathers P, Aftab T. Enhancing artemisinin content in and delivery from Artemisia annua: A review of alternative, classical, and transgenic approaches. Planta. 2021;254(2):29.
9. Taheri A, Almeida-Silva F, Zhang Y, Fu X, Li L, Wang Y, et al. ArtemisiaDB: A comprehensive multi-omics database for Artemisia annua. Plant Commun. 2026;7(7).
10. Judd R, Bagley MC, Li M, Zhu Y, Lei C, Yuzuak S, et al. Artemisinin biosynthesis in non-glandular trichome cells of Artemisia annua. Mol Plant. 2019;12(5):704–14.
11. Zhou L, Huang Y, Wang Q, Guo D, Setzer W. Chromatin Accessibility Is Associated with Artemisinin Biosynthesis Regulation in Artemisia annua. Molecules. 2021;26(4).
12. He W, Liu H, Wu Z, Miao Q, Hu X, Yan X, et al. The AaBBX21–AaHY5 module mediates light-regulated artemisinin biosynthesis in Artemisia annua L. J Integr Plant Biol. 2024;66(8):1735–51.
13. Kayani SI, Yanan M, Fu X, Shen Q, Li Y, Rahman SU, et al. JA-regulated AaGSW1–AaYABBY5/AaWRKY9 complex regulates artemisinin biosynthesis in Artemisia annua. Plant Cell Physiol. 2023;64(7):771–85.
14. Qamar F, Mishra A, Ashrafi K, Saifi M, Dash PK, Kumar S, et al. Increased artemisinin production in Artemisia annua L. by co-overexpression of six key biosynthetic enzymes. Int J Biol Macromol. 2024;281:136291.
15. Zeinali M, Sabzehzari M, Ménard D. A Systematic Review of Alternative Artemisinin Production Strategies. Int J Mol Sci. 2025;26(24):12095.
16. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–21.
17. Hassani D, Taheri A, Fu X, Qin W, Hang L, Ma Y, et al. Elevation of artemisinin content by co-transformation of artemisinin biosynthetic pathway genes and trichome-specific transcription factors in Artemisia annua. Front Plant Sci. 2023;14:1118082.
18. Kim SK, Han GH, Seong W, Kim H, Kim SW, Lee DH, et al. CRISPR interference-guided balancing of a biosynthetic mevalonate pathway increases terpenoid production. Metab Eng. 2016;38:228–40.
19. Das S, Kwon M, Kim JY. Enhancement of specialized metabolites using CRISPR/Cas gene editing technology in medicinal plants. Front Plant Sci. 2024;15:1279738.
20. Koerniati S, Simanjuntak G. CRISPR/Cas9 system for disruption of biochemical pathway for sterol synthesis in Artemisia annua L. IOP Conf Ser Earth Environ Sci. 2020;482(1):012028.
21. Guo C, Xu S, Guo X. Genome-wide analysis of oxidosqualene cyclase genes in Artemisia annua: Evolution, expression, and potential roles in triterpenoid biosynthesis. Curr Issues Mol Biol. 2025;47(7):545.
22. Li Y, Yang Y, Li L, Tang K, Hao X, Kai G. Advanced metabolic engineering strategies for increasing artemisinin yield in Artemisia annua L. Hortic Res. 2024;11(2):uhad292.
23. Tang W, Liu J, Ding B. Nucleic acid nanostructure for delivery of CRISPR/Cas9-based gene editing system. Interdiscip Med. 2023;1(1):e20220014.
24. Fu X, Peng B, Hassani D, Xie L, Liu H, Li Y, et al. AaWRKY9 contributes to light-and jasmonate-mediated to regulate the biosynthesis of artemisinin in Artemisia annua. New Phytol. 2021;231(5):1858–74.
25. Wu Z, Li L, Liu H, Yan X, Ma Y, Li Y, et al. AaMYB15, an R2R3-MYB TF in Artemisia annua, acts as a negative regulator of artemisinin biosynthesis. Plant Sci. 2021;308:110920.
26. Shen Q, Huang H, Xie L, Hao X, Kayani SI, Liu H, et al. Basic helix-loop-helix transcription factors AabHLH2 and AabHLH3 function antagonistically with AaMYC2 and are negative regulators in artemisinin biosynthesis. Front Plant Sci. 2022;13:885622.
27. Xu Z, Li C, Wang X, Lv Z, Li W, Chen W. Transcription factor AabHLH5 participates in JA signaling and negatively regulates artemisinin biosynthesis in Artemisia annua. Physiol Plant. 2025;177(2):e70207.
28. Dong B, Xu Z, Wang X, Li J, Xiao Y, Huang D, et al. TrichomeLess Regulator 3 is required for trichome initial and cuticle biosynthesis in Artemisia annua. Mol Hortic. 2024;4(1):10.

29. Yuan M, Sheng Y, Bao J, Wu W, Nie G, Wang L, et al. AaMYC3 bridges the regulation of glandular trichome density and artemisinin biosynthesis in Artemisia annua. Plant Biotechnol J. 2025;23(2):315–32.
30. Li Y, Chen T, Liu H, Qin W, Yan X, Wu-Zhang K, et al. The truncated AaActin1 promoter is a candidate tool for metabolic engineering of artemisinin biosynthesis in Artemisia annua L. J Plant Physiol. 2022;274:153712.
31. Bao C, Liu F. Prediction of CRISPR on-target effects via deep learning. In: Proceedings of the 1st workshop on NLP for science (NLP4Science). 2024. p. 9–15.
32. Zhang M, Li M, An Y, Liu C, Zhao Q, Zhang D, et al. Single-nucleus transcriptomics reveal the morphogenesis and artemisinin biosynthesis in Artemisia annua glandular trichomes. Nat Commun. 2025;16(1):8646.
33. Gao H, Pei X, Song X, Wang S, Yang Z, Zhu J, et al. Application and development of CRISPR technology in the secondary metabolic pathway of the active ingredients of phytopharmaceuticals. Front Plant Sci. 2025;15:1477894.
34. Xue C, Greene EC. DNA repair pathway choices in CRISPR-Cas9-mediated genome editing. Trends Genet. 2021;37(7):639–56.
35. Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol. 2019;70(1):667–97.
36. Koerniati S, Simanjuntak G. CRISPR/Cas9 system for disruption of biochemical pathway for sterol synthesis in Artemisia annua L. IOP Conf Ser Earth Environ Sci. 2020;482(1):012028.
37. Zaman W, Park S. Advances in Functional Genomics and Biotechnology for Enhancing Therapeutic Potential of Medicinal Plants. Int J Mol Sci. 2026;27(10):4245.
38. Al-Khayri JM, Sudheer WN, Lakshmaiah VV, Mukherjee E, Nizam A, Thiruvengadam M, et al. Biotechnological approaches for production of artemisinin, an anti-malarial drug from Artemisia annua L. Molecules. 2022;27(9):3040.
39. Nath SK. Biotechnological Enhancement of Secondary Metabolites in Medicinal Plants. SARPS J Adv Res Plant Sci. 2025;1(1):16–8.

