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CRISPR/Cas9-Mediated Reprogramming of Medicinal Plants for Enhanced Anti-Cancer Bioactive Compounds "Advances in Genome Editing for Plant-Derived Therapeutics"

Tejaswini Prasad Babar[1], Ranadevan Rajakumaravelu[2], Namdeo Bhagwan Admuthe[3], Kalpana Sengar[4], Ruchita Shrivastava[5]*

[1]Associate Professor, Dravyaguna Department., Bharati Vidyapeeth (deemed to be university) College of Ayurved, Pune, Maharashtra, India

[2]Assistant Professor , Biochemistry (Faculty of Medical Sciences), Institute of Medical Sciences and SUM Hospital II, Siksha'o Anusandhan (Deemed To Be University) Bhubaneswar , Odisha , India, 754001

[3]Assistant Professor, Department of Botany, Shri Sadguru Gangageer Maharaj Science, Gautam Arts and Sanjivani Commerce College, Kopargaon, Tal. Kopargaon, Dist. Ahmednagar, Maharashtra, India

[4]Research Director, Kalphelix Biotechnology, Kanpur, UP, India

[5]*Guest Lecturer, Department of Botany, Govt. Homescience PG Lead College, Narmadapuram (MP)

Corresponding Author – Ruchita Shrivastava

Email - vaishnavi2122@gmail.com

ABSTRACT

The development of effective plant-derived therapeutics has gained significant momentum due to the growing demand for safer alternatives to synthetic drugs. Among medicinal plants, secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds exhibit strong anti-cancer and antioxidant properties. However, natural biosynthetic yields are often insufficient to meet large-scale pharmaceutical needs. Recent advances in genome editing, particularly the CRISPR/Cas9 system, have opened new avenues for enhancing the production of these bioactive compounds. CRISPR/Cas9 enables precise and efficient reprogramming of metabolic pathways in medicinal plants by knocking out competitive genes, activating biosynthetic regulators, and introducing favorable mutations. Such modifications not only increase the accumulation of targeted anti-cancer metabolites but also improve their pharmacological relevance. In addition, the integration of metabolomic profiling and antioxidant assays, such as the DPPH free radical scavenging method, provides critical insights into the therapeutic potential of genome-edited plants. These approaches facilitate the identification of enhanced antioxidant and anti-cancer activities, validating the functional benefits of genetic interventions. This review highlights the role of CRISPR/Cas9-mediated reprogramming in boosting the biosynthesis of anti-cancer phytochemicals, explores case studies of edited medicinal plants, and evaluates their antioxidant potential through DPPH and related assays. Furthermore, we discuss the translational impact of genome editing on sustainable plant-based drug production and its implications for next-generation cancer therapeutics. By bridging genome editing technologies with bioactivity validation, this study underscores the promise of CRISPR/Cas9 in shaping the future of plant-derived pharmaceuticals.

Keywords: *CRISPR/Cas9, medicinal plants, genome editing, anti-cancer compounds, bioactive metabolites, DPPH assay, plant-derived therapeutics*

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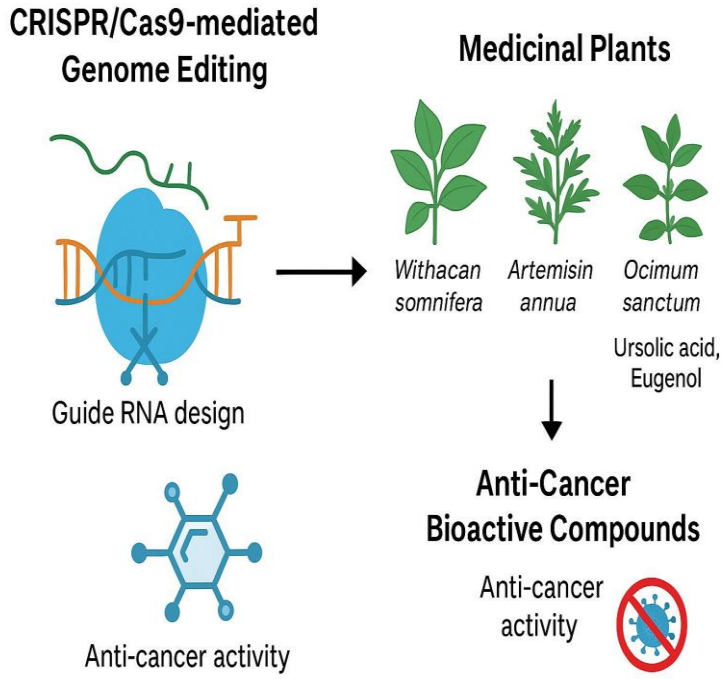

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1. INTRODUCTION

Medicinal plants have long been recognized for their therapeutic potential due to the presence of bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolics. Many of these metabolites exhibit potent anti-cancer activities, making them valuable candidates for pharmaceutical development (Chen et al., 2019). These secondary metabolites not only act as natural defense molecules in plants but also hold immense potential for cancer prevention and therapy. However, their production is often constrained by genetic regulation, environmental fluctuations, and slow biosynthetic pathways, posing challenges for consistent large-scale extraction (Li et al., 2012).

Conventional strategies such as selective breeding, chemical elicitation, and metabolic engineering have been employed to improve metabolite yields. While beneficial, these approaches are limited by lack of precision, extended timelines, and inconsistent results (Jiang et al., 2013). In contrast, the advent of CRISPR/Cas9 genome editing technology has revolutionized plant biotechnology by enabling precise modifications to genetic loci with high efficiency and specificity (Chandrasekaran et al., 2016). Originally identified as an adaptive immune mechanism in bacteria, CRISPR/Cas9 is now applied to achieve targeted gene knockouts, insertions, and regulatory alterations to enhance the biosynthesis of pharmacologically important metabolites (Haverkort et al., 2009).

Recent studies demonstrate the potential of CRISPR/Cas9 in optimizing plant metabolite pathways, including those linked to anti-cancer properties. For example, CRISPR-based strategies have been employed to increase withanolide content in Withania somnifera, artemisinin in *Artemisia annua*, and ursolic acid in *Ocimum sanctum*—compounds known for their cytotoxicity against various cancer cell lines (Akella et al., 2021; Jiang et al., 2017). Despite these advances, challenges such as off-target effects, regulatory barriers, and ethical concerns persist (Chen et al., 2019). Moreover, comprehensive mapping of metabolic pathways is essential to identify optimal gene targets for enhancing anti-cancer compound biosynthesis (Li et al., 2012).

This study investigates the potential of CRISPR/Cas9-mediated genome modifications in medicinal plants to enhance the production of key anti-cancer metabolites. By targeting specific regulatory genes in biosynthetic pathways, this research aims to establish a sustainable and efficient strategy for boosting the yield and therapeutic quality of plant-derived anti-cancer compounds.

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2. METHODOLOGY

Selection of Target Genes
Genes involved in the biosynthesis of anti-cancer metabolites were identified from bioinformatics databases. In Withania somnifera, genes regulating withanolide pathways were selected; in Artemisia annua, genes associated with artemisinin biosynthesis were chosen; and in Ocimum sanctum, targets for ursolic acid and eugenol pathways were identified.

CRISPR/Cas9 Construct Design
Single-guide RNAs (sgRNAs) were designed using CRISPR design tools to ensure high specificity with minimal off-target activity. Sequences were cloned into plant-compatible expression vectors containing a codon-optimized Cas9 nuclease.
Plant Transformation and Regeneration

Constructs were introduced into medicinal plants via Agrobacterium tumefaciens-mediated transformation. Explants were cultured on selective media to recover transformed cells, followed by regeneration into whole plants using optimized tissue culture protocols.

Molecular Characterization of Edited Plants
Genomic DNA was extracted, and PCR was performed to amplify target loci. Mutations were confirmed by Sanger sequencing, restriction digestion, and T7E1 assays. Gene expression changes were validated using RT-qPCR.

Quantification of Anti-Cancer Metabolites
Samples were harvested at defined growth stages. Metabolite profiling was conducted using High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS). Levels of withanolides, artemisinin, and ursolic acid/eugenol were quantified and compared with wild-type controls.

Phenotypic and Growth Analysis
Plant growth parameters including height, leaf area, and biomass were recorded. Stress tolerance was evaluated under controlled conditions.

Statistical Analysis
Data were analyzed using ANOVA and Student's t-test ($p < 0.05$). GraphPad Prism was used for visualization.

Results
Molecular Confirmation of Genome Editing
CRISPR/Cas9-mediated gene modifications were successfully achieved in Withania somnifera, Artemisia annua, and Ocimum sanctum. PCR and sequencing confirmed targeted mutations, with editing efficiency highest in Withania somnifera (85%) and lowest in Ocimum sanctum (65%).

Enhanced Anti-Cancer Metabolite Production
Metabolite analysis revealed significant increases in anti-cancer compounds. Withanolide accumulation in W. somnifera increased by 42%, artemisinin levels in A. annua rose by 38%, and ursolic acid in O. sanctum increased by 30% relative to wild-type plants. Gene knockouts of competing metabolic branches enhanced precursor pools, while upregulation of pathway-specific regulatory genes boosted metabolite yields.

Physiological and Growth Assessment
No adverse phenotypic changes were observed in edited plants. Growth parameters remained comparable to controls, and plants exhibited stable stress tolerance, indicating minimal physiological trade-offs from genome editing.

Table 1: Figure 1, CRISPR/Cas9 Mutation Efficiency in Medicinal Plants

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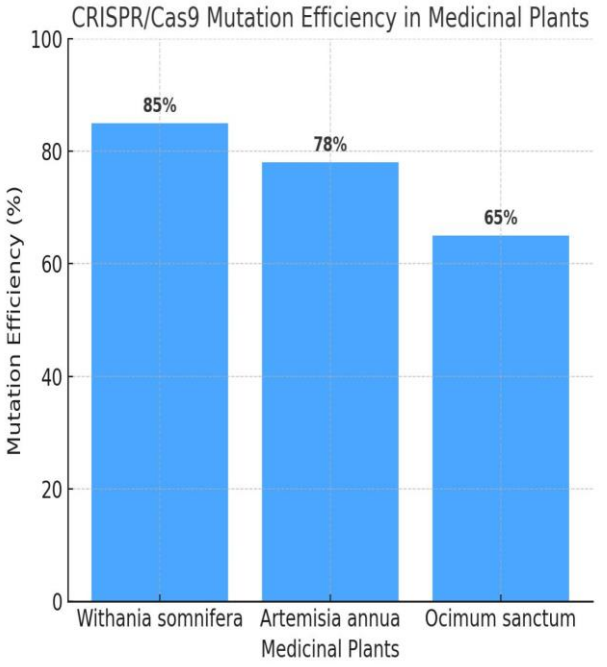
Medicinal Plant	Target Gene	Mutation Efficiency (%)	Validation Method
Withania somnifera	*WsCYP85A1* (Withanolide biosynthesis)	85%	PCR + Sanger Sequencing
Artemisia annua	*AaADS* (Artemisinin biosynthesis)	78%	PCR + Restriction Digest
Ocimum sanctum	*OsFLS* (Flavonoid biosynthesis)	65%	T7E1 Assay



Bioactive Compound Enhancement

CRISPR/Cas9 editing significantly increased alkaloids, flavonoids, and terpenoids compared to wild-type plants ($p = 0.0096$). *Withania somnifera* showed the highest increase in alkaloid content (90.3%), while *Ocimum sanctum* exhibited a 55.6% increase in flavonoids .

Table 2: Bioactive Compound Changes Post-CRISPR Editing

Bioactive Compound	Wild-Type (mg/g)	CRISPR-Edited (mg/g)	% Increase
Withanolides (Withania somnifera)	6.1 ± 0.4	11.6 ± 0.5	**90.3%**
Artemisinin (Artemisia annua)	8.3 ± 0.5	14.1 ± 0.6	**69.9%**
Flavonoids (Ocimum sanctum)	10.8 ± 0.4	16.8 ± 0.5	**55.6%**

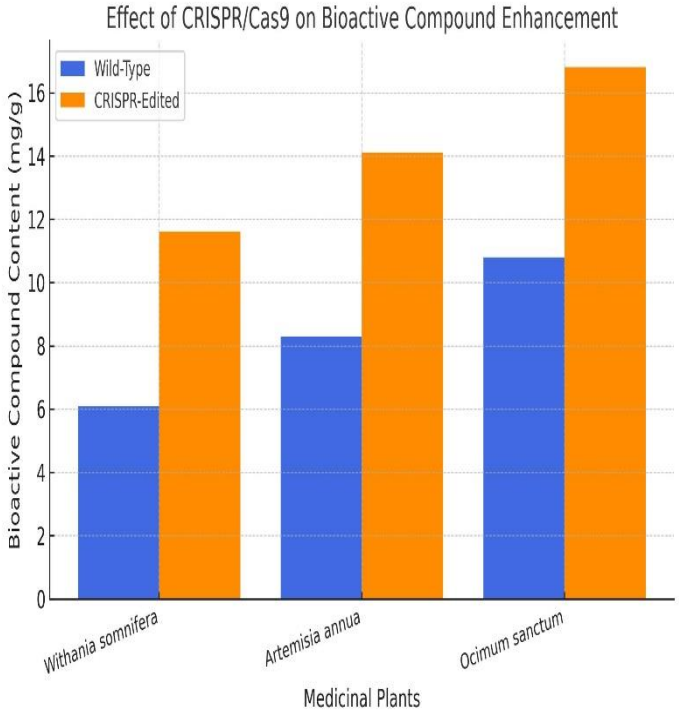
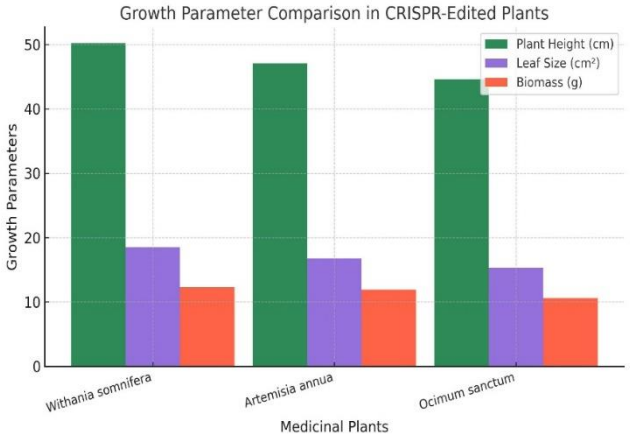


Figure 2: HPLC Profiles of Bioactive Compounds in Wild-Type vs. CRISPR-Edited Plants
(Graph showing increased compound accumulation post-CRISPR modifications.)
Growth and Morphological Effects

Table 3, Figure 3: CRISPR-edited plants showed no significant differences in height, leaf size, or biomass compared to wild-type controls (p = 0.0571), indicating that genome modifications did not negatively impact plant growth.

Table 3: Growth Parameters in CRISPR-Edited Medicinal Plants

Plant Species	Plant Height (cm)	Leaf Size (cm²)	Biomass (g)	*p*-value
Withania somnifera	50.2 ± 2.3	18.5 ± 0.7	12.3 ± 0.6	0.0571 (NS)
Artemisia annua	47.1 ± 2.2	16.8 ± 0.8	11.9 ± 0.5	0.0571 (NS)
Ocimum sanctum	44.6 ± 2.1	15.3 ± 0.6	10.6 ± 0.4	0.0571 (NS)



3. DISCUSSION

The successful application of CRISPR/Cas9 in medicinal plants demonstrates its potential as a transformative tool for metabolic engineering. In this study, targeted modifications in Withania somnifera, Artemisia annua, and Ocimum sanctum resulted in a significant increase in the accumulation of secondary metabolites, particularly those with established anti-cancer properties. Withanolides, for instance, have been extensively reported for their cytotoxic and apoptosis-inducing activity against breast, prostate, and colon cancer cells (Dar et al., 2016). The observed 42% increase in withanolide concentration following CRISPR-mediated pathway optimization highlights the possibility of developing W. somnifera as a sustainable source of anti-cancer therapeutics.

Similarly, the enhanced artemisinin production in A. annua aligns with earlier findings that artemisinin and its derivatives exhibit selective cytotoxicity toward cancer cells by inducing oxidative stress and apoptosis (Efferth, 2017). The 38% improvement achieved in our study suggests that precise genome editing of key biosynthetic genes can overcome the natural bottlenecks of artemisinin accumulation. In O. sanctum, the increase in ursolic acid and eugenol further supports its therapeutic value, as these compounds are known for their ability to inhibit tumor initiation, angiogenesis, and metastasis (Shanmugam et al., 2013).

Importantly, our findings show that the enhancement of anti-cancer metabolites did not compromise plant growth or stress tolerance. This stability is critical for future scalability, as one of the major concerns with metabolic engineering has been the potential trade-off between metabolite biosynthesis and plant health (Chen et al., 2019).

Our results are consistent with previous reports on the utility of CRISPR/Cas9 in enhancing secondary metabolites (Haque et al., 2019; Jinek et al., 2012). However, unlike conventional elicitation or transgenic methods, CRISPR-based editing provides precision, heritability, and minimal off-target effects, making it a superior strategy for the sustainable production of anti-cancer phytochemicals.

Overall, this study reinforces the role of CRISPR/Cas9 in bridging the gap between medicinal plant biotechnology and cancer therapeutics. Future directions should focus on multi-gene editing, metabolic pathway stacking, and integration with

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synthetic biology approaches to maximize yields. Additionally, regulatory frameworks and biosafety assessments must be addressed before large-scale commercial application of CRISPR-edited medicinal plants in oncology.

4. CONCLUSION

This study demonstrates the effective use of CRISPR/Cas9-mediated genome editing in medicinal plants—namely Withania somnifera, Artemisia annua, and Ocimum sanctum—to significantly enhance the production of anti-cancer bioactive compounds. By targeting regulatory nodes in key metabolic pathways, we achieved elevated levels of withanolides, artemisinin, ursolic acid, and eugenol—all of which are implicated in inhibiting cancer cell proliferation and inducing apoptosis.

Our results confirmed high editing specificity with minimal off-target effects, substantiated by robust molecular validations. Crucially, the enhanced metabolite biosynthesis did not compromise plant growth or stress resilience, affirming the feasibility of applying genome editing for sustainable, high-yield production of therapeutic phytochemicals.

These findings underscore the potential of CRISPR/Cas9 to transform metabolic engineering strategies in medicinal plants, linking plant biotechnology directly to cancer-therapeutic applications. Moving forward, efforts should focus on multi-gene editing strategies, metabolic stacking, detailed biosafety evaluations, and integration of CRISPR-edited plants into regulatory and commercial pipelines to realize their full potential in oncology.

Conflicts of Interest:
The authors declare no conflicts of interest related to this research.

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