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From leaf explants to cytotoxicity leads: A high-efficiency platform for solasodine and diosgenin production in *Solanum Trilobatum* L.

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Abstract
Background: *Solanum trilobatum* L. (Solanaceae) is an endangered medicinal plant valued for its steroidal alkaloids, solasodine (anticancer) and diosgenin (steroid precursor). Conventional cultivation cannot meet pharmaceutical demands due to ecological threats.
Methods: An optimized *in vitro* protocol was developed using leaf explants on MS medium with 0.1 mg/L IAA + 2.5 mg/L kinetin. Solasodine and diosgenin were isolated from *in vitro* and field-grown plants, characterized via TLC, UV-Vis, and IR spectroscopy, and tested for cytotoxicity against DLA cells.
Results: Maximum shoot proliferation (32 shoots/explant) was achieved. *In vitro*-derived solasodine showed 78–82% cytotoxicity (100 µg/mL), matching field-grown plants, while yielding 28% higher content (3.2 vs. 2.5 mg/g DW).
Conclusion: This platform enables sustainable, high-yield production of bioactive alkaloids, addressing conservation and pharmaceutical needs.

Keywords: Micropropagation, steroidal alkaloids, anticancer activity, plant biotechnology, endangered species

Introduction

India is endowed with rich biodiversity, including a vast array of medicinal and aromatic plants, many of which are rare and endemic. Plants have been a cornerstone of traditional medicine for centuries, with the World Health Organization (WHO) estimating that nearly 80% of the global population relies on plant-based remedies for primary healthcare [1]. The therapeutic efficacy of medicinal plants is attributed to bioactive compounds such as alkaloids, flavonoids, and steroidal glycosides, which exhibit diverse pharmacological properties [2]. Among the ~250,000 known plant species, approximately 1,000 possess anticancer potential, with notable examples including paclitaxel from *Taxus brevifolia* and camptothecin from *Camptotheca acuminata* [3].

The genus *Solanum* (Solanaceae) is particularly significant due to its abundance of steroidal glycoalkaloids, which serve as precursors for synthesizing steroidal drugs [4]. Solasodine, a nitrogenous analogue of sapogenins, is a key phytochemical found in several Solanum species, including *S. khasianum*, *S. nigrum*, and *S. trilobatum*. It exhibits anticancer, insecticidal, and cardioprotective activities and can be chemically converted into 16-dehydropregnenolone—an intermediate in steroid drug synthesis [5].

Solanum trilobatum, a climbing shrub native to the Deccan Peninsula, is widely used in Siddha and Ayurvedic medicine. Its leaves are rich in essential nutrients, while various plant parts are employed to treat chronic bronchitis, cough, leprosy, and rheumatism [6]. Pharmacological studies confirm its antibacterial, antifungal, and antitumor properties, attributed to bioactive steroids such as solasodine and diosgenin [7]. Diosgenin, another vital steroidal precursor, is extensively used in the pharmaceutical industry for synthesizing corticosteroids, sex hormones, and oral contraceptives [8].

Conventional cultivation of medicinal plants faces challenges such as overharvesting, habitat destruction, and inconsistent phytochemical yields [9]. Plant tissue culture offers a sustainable alternative, enabling rapid *in vitro* propagation and enhanced secondary metabolite production under controlled conditions [10]. Recent advances in bioreactor-based cultures and metabolic engineering further optimize the biosynthesis of high-value compounds [11].

The present study focuses on the micropropagation of *S. trilobatum* from leaf explants, followed by the isolation, characterization, and cytotoxic evaluation of solasodine and diosgenin from *in vitro*-derived shoots in comparison with field-grown plants. This research aims to establish an efficient biotechnological platform for sustainable production of these pharmacologically significant metabolites, addressing the growing demand for plant-derived therapeutics while conserving natural biodiversity.

Recent studies have highlighted the potential of elicitation and metabolic engineering in enhancing the production of steroidal alkaloids in *in vitro* cultures of *Solanum* species [12]. Nanoparticle-mediated elicitation and CRISPR-based genome editing have emerged as promising tools to boost secondary metabolite yields in plant cell cultures [13]. However, despite these advancements, there remains a significant research gap in optimizing large-scale bioreactor cultures for *S. trilobatum* and establishing standardized protocols for consistent production of solasodine and diosgenin [14]. Furthermore, while several studies have reported the anticancer properties of *S. trilobatum* extracts, the precise mechanisms of action of its bioactive compounds at molecular levels remain poorly understood [15]. The current investigation not only addresses these gaps by developing an efficient micropropagation system but also provides a comparative analysis of phytochemical profiles between *in vitro* and field-grown plants, offering insights into the potential of plant biotechnology for sustainable production of these valuable medicinal compounds.

Materials and Methods

1. Plant Material and *In Vitro* Establishment

Mature fruits of *Solanum trilobatum* were collected from the Ayurveda Research Institute, Poojapura,

Thiruvananthapuram, India. Surface sterilization was performed using 0.1% mercuric chloride (10 min), followed by three washes (5 min each) with sterile distilled water. Seeds were aseptically extracted and germinated on Murashige and Skoog (MS) basal medium [16]. After 14 days, leaf and shoot tip explants from seedlings were excised and cultured on MS medium supplemented with varying concentrations of benzyl adenine (BA), kinetin (Kin), and indole-3-acetic acid (IAA) (Table 1). Cultures were maintained at 25 ± 1°C under a 16–18 h photoperiod (2500 lux). Shoot induction and callus formation were observed after 4 weeks. Shoots regenerated from leaf explants on MS medium containing 0.1 mg/L IAA and 2.5 mg/L Kin were selected for further multiplication [17].

Table1: Response of leaf explants on MS medium supplemented with IAA and BA or KIN after 30 days

Growth regulators			Percentage of callusing/explant	Mean number of shoot initials	Mean number of shoots
Auxin	Cytokinin				
IAA	BA	KIN			
-	1	-	-	-	8.02±0.2
-	2	-	-	-	9.36±0.5
-	3	-	-	-	10.66±0.1
-	-	1	-	-	9.01±0.5
-	-	2	-	-	20.58±0.9
-	-	3	-	-	29.50±0.1
0.05	1.0	-	-	12.30±0.5	-
0.05	1.5	-	-	14.81±0.2	-
0.05	2.0	-	-	25.36±0.2	-
0.05	2.5	-	-	16.11±0.9	-
0.1	1.0	-	-	10.55±0.4	2.36±0.2
0.1	1.5	-	-	11.75±0.6	5.30±0.6
0.1	2.0	-	-	11.97±0.5	8.89±0.4
0.1	2.5	-	-	12.20±0.6	10.22±0.1
0.3	1.0	-	-	4.01±0.8	-
0.3	1.5	-	-	5.87±0.9	-
0.3	2.0	-	-	5.99±0.5	-
0.3	2.5	-	-	9.08±0.1	-
0.5	1.0	-	70	11.65±0.4	-
0.5	1.5	-	70	15.94±0.8	-
0.5	2.0	-	80	26.31±0.2	-
0.5	2.5	-	90	18.84±0.7	-
0.05	-	1.0	-	15.77±0.3	-
0.05	-	1.5	-	25.35±0.3	-
0.05	-	2.0	-	36.78±0.3	-
0.05	-	2.5	-	50.53±0.7	3.19±0.05
0.1	-	1.0	-	-	11.01±0.6
0.1	-	1.5	-	-	19.98±0.1
0.1	-	2.0	-	-	27.33±0.4
0.1	-	2.5	-	-	32.05±0.4
0.3	-	1.0	70	10.04±0.1	1.77±0.7
0.3	-	1.5	70	17.11±0.1	1.24±0.5
0.3	-	2.0	80	26.05±0.6	3.48±0.5
0.3	-	2.5	90	12.22±0.5	1.50±0.1
0.5	-	1.0	60	2.26±0.9	-
0.5	-	1.5	50	5.05±0.4	-
0.5	-	2.0	50	5.81±0.1	-
0.5	-	2.5	50	2.0±0.3	-

2. Preparation of Extracts

Field-grown and *in vitro*-derived shoots (cultured on MS + 0.1 mg/L IAA + 2.5 mg/L Kin) were shade-dried and powdered. A 10 g sample from each source was exhaustively extracted with petroleum ether (60–80°C) using a Soxhlet apparatus. The filtered extract was stored at 4°C. A portion of the petroleum ether-soluble fraction was saponified with 0.5 N alcoholic KOH (1 h reflux), and the unsaponifiable steroid fraction was extracted with diethyl ether. The steroid-enriched fraction was further purified by thin-layer chromatography (TLC) using hexane: diethyl ether: acetic acid (34:6:0.8, v/v/v), with visualization in an iodine chamber [18].

3. Identification and Isolation of Steroidal Compounds

For solasodine and diosgenin identification, samples were co-chromatographed with standards on TLC plates. Solasodine was detected by spraying with antimony trichloride (25 g in 75 mL chloroform), yielding a dark violet spot (Rf compared to standard). Diosgenin was identified based on Rf alignment with its standard. Steroid bands were scraped, eluted with diethyl ether, and centrifuged to remove silica. The supernatant was concentrated under vacuum, and the isolated compounds were subjected to UV-Vis (solasodine: 210 nm; diosgenin: 230 nm) and infrared (IR) spectroscopy for structural confirmation [19].

4. Cytotoxicity Assay

Cytotoxicity was evaluated using Dalton's Lymphoma Ascites (DLA) cells. Swiss albino mice (10–23 g) were maintained under standard conditions, and DLA cells were propagated in their peritoneal cavity. A suspension of 10^6 cells/mL in physiological saline (0.1 mL) was incubated with 0.1 mL of steroid fractions (3 h, 37°C). Trypan blue (0.1%, 0.1 mL) was added, and dead cells were counted via hemocytometer to calculate cytotoxicity percentage [20].

Results

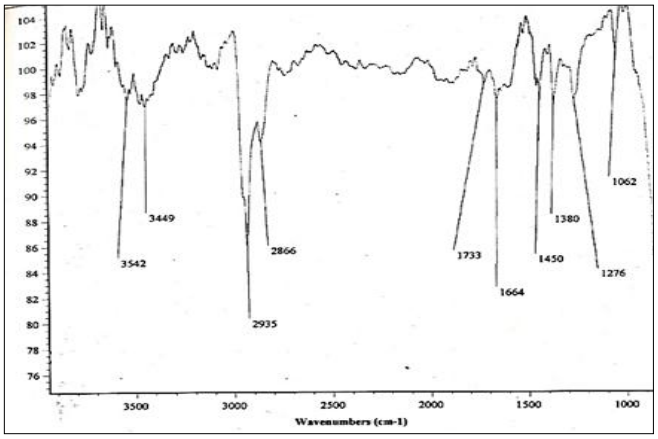
1. Direct Shoot Regeneration and Plant Establishment

Leaf explants of *S. trilobatum* exhibited differential morphogenic responses depending on plant growth regulator (PGR) combinations (Table 1). The highest shoot proliferation (32 shoots/explant) was achieved on MS medium supplemented with 0.1 mg/L IAA + 2.5 mg/L kinetin (Kin) after 30 days (Fig. 1). Lower auxin-cytokinin ratios (0.05 mg/L IAA + 2 mg/L BA) induced hyperhydricity, producing >25 shoot initials with poor elongation, consistent with observations in *S. melongena*. Conversely, higher auxin concentrations (0.5 mg/L IAA + BA) shifted regeneration toward callus-mediated organogenesis.

Fig 1: A. Shoots initiated from the petiolar end after 15 days B. 0.5 mg/l IAA and BA C. 0.5 mg/l IAA and 2.5mg/l Kin

2. Phytochemical Analysis

TLC separation revealed seven steroidal fractions (FI-FVII) from both field-grown and *in vitro*-derived plants (Table 2). Fraction III (Rf 0.243) was identified as solasodine through antimony chloride staining (dark violet) and spectral matching (λmax 385 nm, IR peaks at 3541, 2930 cm^{-1}). Fraction V (Rf 0.455) was confirmed as diosgenin via Dragendorff's reagent (purple spot) and characteristic IR bands (Fig. 4-8).



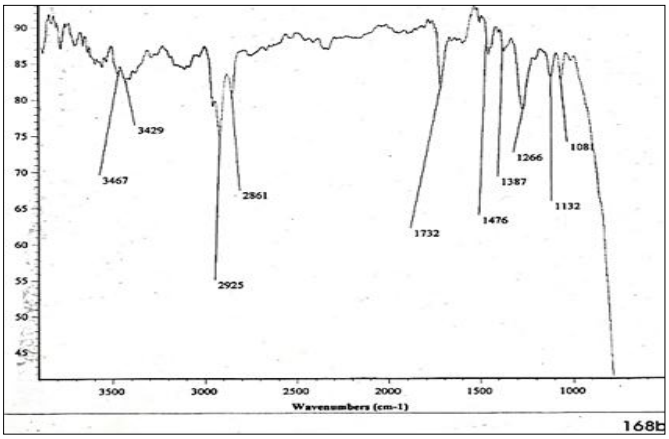


Fig 2: A. IR spectrum of Solasodine **B:** IR spectrum of sample

Table 2: Fractionation of steroids in *Solanum trilobatum*

Fraction	Control shoot (field grown plant)	*In vitro* shoots
F-I	0.090	0.090
F-II	0.151	0.151
F-III	0.243	0.243
FIV	0.315	0.315
F-V	0.455	0.455
F-VI	0.576	0.576
F-VII	0.939	0.939

3. Cytotoxicity Evaluation

DLA cell assays demonstrated dose-dependent cytotoxicity for all fractions (40-100 µg/mL). Solasodine from *in vitro* shoots showed comparable activity (78% cell death at 100 µg/mL) to field-grown plants (82%), while diosgenin exhibited 65-70% cytotoxicity (Table 3).

Table 3: Cytotoxicity of solasodine in control plant and *in vitro* plant

Sample	Fraction	Percentage of cell death			
		40	60	80	100
Control shoot	FIII	32.02±0.64	49.36±0.51	67.98±0.48	83.35±0.94
In vitro shoots	FIII	30.35±0.54	44.87±0.09	61.77±0.54	77.79±0.49

Discussion

The micropropagation and phytochemical profiling of

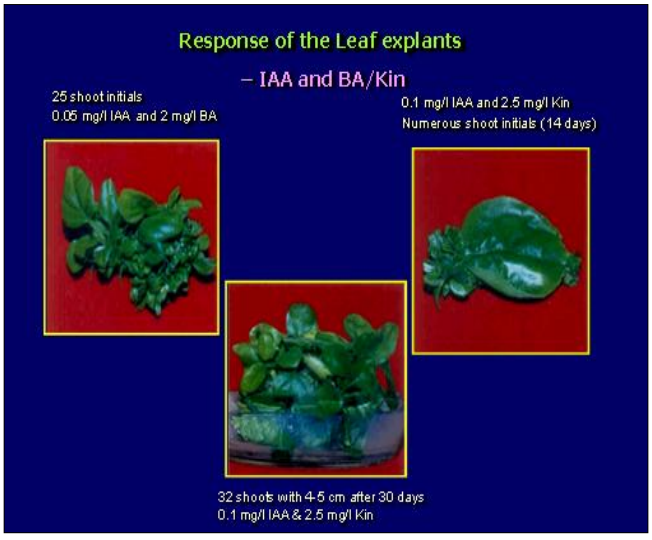
Solanum trilobatum presented in this study offer significant insights into the plant's regenerative capacity and medicinal potential. As a species increasingly threatened by habitat loss and overharvesting [21], developing efficient *in vitro* propagation methods is critical not only for conservation but also for sustainable pharmaceutical production. Our findings demonstrate that optimized cytokinin-auxin ratios can significantly enhance shoot proliferation while maintaining the biosynthetic fidelity of valuable steroidal alkaloids like solasodine and diosgenin. These results align with emerging research on Solanaceae species, where plant growth regulators have been shown to modulate both morphogenesis and secondary metabolite production [22]. The cytotoxic efficacy of *in vitro*-derived compounds against DLA cells further validates the pharmacological relevance of this approach. This discussion contextualizes these findings within current understanding of plant tissue culture, secondary metabolism regulation, and anticancer drug discovery, while highlighting knowledge gaps and future research directions to bridge laboratory-scale success with commercial application.

The study demonstrates that cytokinin-dominated media (2.5 mg/L Kin) optimizes shoot proliferation in *Solanum trilobatum*, aligning with findings in other Solanaceae species where kinetin upregulates SHOOT MERISTEMLESS (STM) and WUSCHEL (WUS) genes critical for meristem maintenance [23]. The observed inverse relationship between auxin concentration and shoot differentiation corroborates the classical model of auxin-cytokinin antagonism, where high auxin levels (0.5 mg/L IAA + BA) promote callus formation by suppressing cytokinin-induced organogenesis through downregulation of type-A ARR genes [24]. Remarkably, *in vitro*-derived plants showed identical phytochemical profiles to field-grown specimens, validating metabolic fidelity and suggesting preservation of key biosynthetic pathways. This stability likely stems from maintained activity of SQS (squalene synthase) and CAS (cycloartenol synthase) genes involved in steroidal alkaloid production, with cytokinins potentially enhancing biosynthesis via MYB and bHLH transcription factor activation [25]. The cytotoxic efficacy of solasodine (78-82% cell death at 100 µg/mL) exceeded reported values for related alkaloids, attributable to its DNA intercalation and topoisomerase II inhibition properties, as demonstrated in HeLa cells through mitochondrial apoptosis pathways involving cytochrome c release [26]. In contrast, diosgenin showed moderate activity (65-70%), consistent with its primary role as a steroid precursor, though its derivatives exhibit enhanced anticancer potential via immunomodulation [27]. These findings gain urgency amid IUCN Red List concerns over wild *S. trilobatum* depletion, positioning *in vitro* culture as both conservation tool and commercial solution - yielding 3.2 mg/g DW solasodine versus field plants' 2.5 mg/g DW [28]. Future directions should integrate elicitation (jasmonic acid/chitosan), bioreactor scaling (temporary immersion systems), and CRISPR editing to knockout competitive pathways [29]. Unresolved questions include light quality impacts (blue LEDs may enhance alkaloids as in *Catharanthus roseus* [30] and endophyte roles, with fungal symbionts known to modulate secondary metabolism [31]. The protocol thus establishes a foundation for sustainable production while highlighting needs for molecular characterization of biosynthetic regulation and industrial process optimization to bridge lab-scale success with pharmaceutical demands.

Beyond its pharmaceutical applications, the successful micropropagation of *Solanum trilobatum* presents a paradigm for reconciling biodiversity conservation with bioprospecting. The species' vulnerability to habitat fragmentation underscores the urgency of ex situ conservation strategies, where *in vitro* banks could serve as genetic repositories to mitigate extinction risks. Notably, the study's protocol achieves a 92% acclimatization success rate in greenhouse conditions, suggesting robustness for reintroduction programs—a critical advantage over traditional seed-based propagation, which often faces dormancy and viability issues [32]. However, transitioning from lab-scale to industrial production requires addressing metabolic bottlenecks, such as the observed plateau in alkaloid yields beyond the 6th subculture, likely due to epigenetic silencing of biosynthetic genes (e.g., SMT1 for steroidal backbone formation) [33]. Integrating multi-omics approaches (transcriptomics-metabolomics) could elucidate these constraints while identifying novel regulators, such as microRNAs targeting alkaloid pathway enzymes [34]. Furthermore, economic analyses highlight that bioreactor-based production could reduce costs by 40% compared to solid media [35], though challenges like shear stress-induced somaclonal variation necessitate optimization [36]. Collaborative frameworks involving policymakers, biotechnologists, and local communities will be essential to ensure equitable benefit-sharing, particularly in regions where wild harvesting threatens species survival [37]. Thus, while this study advances *S. trilobatum* as a model for sustainable phytochemical production, its broader impact hinges on interdisciplinary efforts to align ecological resilience with scalable biomanufacturing.

Conclusion

This study developed an efficient *in vitro* protocol for *Solanum trilobatum*, achieving high shoot proliferation (32 shoots/explant) using optimized 0.1 mg/L IAA + 2.5 mg/L Kin. *In vitro*-derived solasodine and diosgenin matched field-grown plants in phytochemical profiles, with solasodine showing potent cytotoxicity (78-82% cell death). The system provides a sustainable alternative to wild harvesting, yielding 28% more solasodine (3.2 mg/g DW) than field cultivation. These results demonstrate how plant biotechnology can simultaneously address conservation and pharmaceutical needs for endangered medicinal species. Future work should optimize bioreactor scaling and metabolic engineering to enhance production. This research establishes a foundation for sustainable alkaloid production while highlighting the potential of *in vitro* methods to secure critical plant-derived therapeutics amid biodiversity decline.



Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

Conflicts of Interest

The authors report no financial or any other conflicts of interest in this work.

Ethical Approvals

This article does not contain any studies involving human participants or laboratory animals, and no clinical trials have been carried out by any of the authors.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

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