World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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Optimization of explant type and auxin-cytokinin combinations for enhanced organogenesis, solasodine accumulation, and cytotoxicity in *Solanum trilobatum* L.

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World Journal of Biology Pharmacy and Health Sciences, 2026, 26(02), 249-258

Publication history: Received on 17 March 2026; revised on 02 May 2026; accepted on 05 May 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.26.2.0239

Abstract

Solanum trilobatum L. is a medicinal climber valued for its steroidal alkaloids, particularly solasodine, but its natural propagation is limited. This study established a high-frequency regeneration protocol by comparing shoot tip, node, petiole, and leaf explants on MS medium with various auxin-cytokinin combinations. Leaf explants on 0.1 mg/L IAA + 2.5 mg/L Kin produced the highest shoots (32/explant). Friable callus induced on 2 mg/L 2,4-D was used to establish suspension cultures (PCV 0.756 mL/mL). Biochemical analysis revealed maximum carbohydrates in *in vitro* shoots (10.623 mg/g FW) and peak proteins in flowering *in vivo* shoots (12.654 mg/g FW). Phytochemical analysis showed that control and *in vitro* shoots contained seven steroid fractions including solasodine (1.107 and 1.007 mg/g respectively), which was absent in *in vivo* shoots. Cytotoxicity assays against DLA cells demonstrated that total steroid fractions from *in vitro* shoots caused 100% cell death at 80 µg, with CC_{50} values of 22.5 µg (diosgenin) and 21.0 µg (betulin). Hardened plantlets achieved 80% field survival. The loss of solasodine during acclimatization suggests that direct harvesting of *in vitro* shoots is preferable for steroidal alkaloid production, and the cytotoxic equivalence validates micropropagation for producing therapeutically potent biomass.

Keywords: *Solanum trilobatum*; Micropropagation; Solasodine; Cytotoxicity; DLA cell lines; Leaf explant

1. Introduction

Solanum trilobatum L. (Solanaceae), commonly referred to as the "Purple Fruited Pea Eggplant," is a significant medicinal climber indigenous to the tropical regions of India and Southeast Asia (Kumar et al., 2010). This species holds a prestigious position in traditional Siddha and Ayurvedic medicine, where it is utilized as a primary therapeutic agent for managing respiratory conditions such as bronchial asthma, chronic bronchitis, and tuberculosis (Mohan and Kalidass, 2013). Modern pharmacological evaluations have confirmed its efficacy as a hepatoprotective, anti-inflammatory, and antioxidant agent (Shahjahan et al., 2005), with recent research increasingly focusing on its potent anti-cancer properties against various cell lines (Muthu and Michael, 2012). These diverse bioactivities are primarily attributed to a rich reservoir of secondary metabolites, particularly steroidal alkaloids such as solasodine (Patel and Rao, 2014). Solasodine is a critical industrial precursor for the semi-synthesis of various steroidal drugs, including corticosteroids, oral contraceptives, and sex hormones (Bhat et al., 2012).

Despite its immense pharmaceutical value, the natural cultivation of *S. trilobatum* faces several bottlenecks. Propagation via seeds is frequently hampered by poor germination rates and inherent seed dormancy (Josekutty and Prathapasenan, 2012). While traditional vegetative methods exist, they are often too slow to meet escalating industrial demands. Furthermore, wild populations are under increasing pressure due to habitat loss and over-harvesting, which threatens the species' biodiversity (Rani and Rana, 2015). Consequently, plant tissue culture (PTC) has emerged as an essential tool for rapid, large-scale clonal multiplication and the standardized production of secondary metabolites under controlled conditions (Ramachandra Rao and Ravishankar, 2002).

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There remains a significant gap in the literature regarding the comparative optimization of varied explant types—specifically shoot tips, nodes, leaves, and petioles—under diverse auxin-cytokinin ratios to achieve both high-frequency organogenesis and maximum secondary metabolite accumulation (Saritha et al., 2007). While some studies have explored basic micropropagation of *Solanum* species (Sridhar and Naidu, 2011), there is a lack of comprehensive data bridging the transition from *in vitro* multiplication to the functional bio-equivalence of these plantlets compared to field-grown counterparts (Jain and Nessler, 2016). The significance of this study lies in establishing a high-frequency regeneration protocol that ensures not only morphological fidelity but also chemical consistency. By identifying the most responsive explants and hormonal combinations, this research facilitates the sustainable production of *S. trilobatum* biomass. Crucially, the study provides a critical assessment of biochemical content (carbohydrates and proteins) and quantifies solasodine across different tissues, validating the therapeutic potency of *in vitro*-derived materials through cytotoxicity assays against DLA (Dalton's Lymphoma Ascites) cell lines (Sundaram et al., 2019).

2. Materials and Methods

2.1. Plant Material and Surface Sterilization

Solanum trilobatum Linn. seedlings were procured from the Ayurveda Research Institute, Thiruvananthapuram, and maintained in the garden of the Department of Botany, University of Kerala (Kumar et al., 2010). Fruits were washed with 1% Labolene detergent under running tap water for 20–30 min, followed by surface sterilization with 0.1% mercuric chloride for 10 min inside a laminar air flow cabinet. After thorough rinsing with sterile distilled water, seeds were aseptically dissected and germinated on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962). Cultures were incubated at 25±2°C under a 16 h photoperiod (2500 lux) and 50–65% relative humidity (Josekutty and Prathapasenan, 2012).

2.2. Explant Inoculation and Callus Induction

Shoot tips, nodes, leaves, and petioles excised from in vitro seedlings were inoculated onto MS medium supplemented with various concentrations of cytokinins (BA and Kin: 1–3 mg/L alone or 0.5–2.5 mg/L in combination) and auxins (IAA, NAA, 2,4-D: 0.05–3 mg/L alone or in combination) (Saritha et al., 2007). Calli induced from leaf explants on 2 mg/L 2,4-D alone and on 1 mg/L 2,4-D + 1.5 mg/L Kin were selected for long-term maintenance and subcultured every 45 days (Ramachandra Rao and Ravishankar, 2002).

2.3. Shoot Regeneration and Multiplication

Shoots regenerated from leaf explants on MS medium supplemented with 0.1 mg/L IAA and 2.5 mg/L Kin exhibited the best regeneration response and were selected for multiplication. These shoots were subcultured on the same medium at 45-day intervals and maintained as stock cultures for further experiments (Patel and Rao, 2014).

2.4. Rooting, Hardening and Acclimatization

Healthy shoots (4–5 cm) were transferred to MS medium supplemented with IAA, NAA, or IBA (0.5–2 mg/L alone or in combination) for root induction (Bhat et al., 2012). Rooted plantlets were transferred to sterile vermiculite in plastic cups, nourished with half-strength liquid MS medium, and covered with polythene bags to maintain humidity. After 7 days, bags were gradually opened, and plantlets were acclimatized to room temperature. They were then transplanted to pots containing compost and sand (1:3) and maintained in shade for two weeks before transfer to the field (Muthu and Michael, 2012).

2.5. Cell Suspension Culture

Approximately 500 mg of friable callus derived from leaf explants on 2 mg/L 2,4-D was transferred to 150 mL liquid MS medium containing 2 mg/L 2,4-D and incubated on a gyratory shaker at 100–150 rpm under a 16 h photoperiod. After 60 days, the culture was filtered through 350 µm nylon gauze, and packed cell volume was determined (Dodds and Roberts, 1985). Harvested cells and filtered medium were processed separately for steroid extraction (Rani and Rana, 2015).

2.6. Biochemical Analysis of Primary Metabolites

Six samples were analyzed: field-grown shoots (control), in vitro shoots (0.1 mg/L IAA + 2.5 mg/L Kin), 5-month-old *in vivo* shoots, 12-month-old flowering shoots, pale yellow callus (2 mg/L 2,4-D), and pale brown friable callus (1 mg/L 2,4-D + 1.5 mg/L Kin). Total carbohydrates were estimated using the anthrone method (Hedge and Hofreiter, 1962),

and total proteins were quantified using the Lowry method (Lowry et al., 1951). Protein profiles were analyzed by SDS-PAGE according to standard protocols (Laemmli, 1970).

2.7. Phytochemical Analysis and Steroid Quantification

Petroleum ether (60–80°C) extracts were prepared from dried and powdered samples of shoots, roots, callus, and suspension culture cells using a Soxhlet apparatus. The extracts were saponified with 0.5 N alcoholic KOH, and the unsaponifiable steroid-rich fractions were recovered by diethyl ether extraction (Mohanan et al., 1998). Analytical TLC was performed on silica gel G 60 plates using a solvent system of hexane:diethyl ether:acetic acid (85:15:2, v/v). Steroidal components were identified using iodine vapor, antimony trichloride (for solasodine), Libermann-Burchard reagent (for β-sitosterol), and Dragendorff's reagent (for diosgenin) (Emke and Eilert, 1986). The isolated fractions were purified by scraping and elution with diethyl ether. Quantification of steroids was performed by UV-Vis spectrophotometry at 210, 230, and 385 nm, and structural confirmation was carried out using infrared (IR) spectroscopy (Shahjahan et al., 2005).

2.8. *In Vitro* Cytotoxicity Assay

The cytotoxicity of various extracts was evaluated against Dalton's Lymphoma Ascites (DLA) cells maintained in Swiss albino mice (10–23 g body weight) by intraperitoneal injection of 1×10^6 cells (Muthu and Michael, 2012). Aqueous decoctions, petroleum ether extracts, total steroid fractions, and TLC-purified fractions from shoots, roots, and callus were tested. DLA cells (1×10^6 cells/0.1 mL) were incubated with varying concentrations of each extract at 37°C for 3 h. Cell viability was assessed using the trypan blue (0.1%) exclusion method, and the percentage of dead cells was calculated. The concentration required for 50% cytotoxicity (CC_{50}) was determined from dose-response curves (Sundaram et al., 2019).

3. Results

3.1. Explant Response and Organogenesis

Observations on shoot tip, petiole, node, and leaf explants were recorded 30 days after inoculation on MS medium with various auxin-cytokinin combinations. Responses varied significantly among explant types and hormonal treatments.

3.1.1. Shoot Tip Explants

Shoot tips on BA or Kin alone (1–3 mg/L) produced 3–5 shoots. The highest regeneration (>25 shoot initials) was observed on 0.1 mg/L IAA + 2.5 mg/L Kin. On 0.05 mg/L IAA + 2 mg/L BA, five shoots (3–4 cm) with one root were produced. NAA-containing media generally yielded single shoots, while 2,4-D predominantly induced callus proliferation rather than shoots (Table 1).

Table 1 Results of shoot tip explant response to selected hormonal combinations

Hormone combination (mg/L)	No. of shoots	Rooting	Callus response
Kin 3 (alone)	5	-	Minimal
IAA 0.05 + BA 2	5	Single root	Minimal
IAA 0.1 + BA 2	4 (14 initials)	-	Minimal
IAA 0.1 + Kin 2.5	>25 initials	-	Minimal
NAA 0.3 + BA 1	1	Single root	Minimal
2,4-D 0.5 + BA 2	Dwarf shoots	-	Profuse nodular
2,4-D 0.1 + Kin 2.5	None	-	Green solid

3.1.2. Petiole Explants

Petiole explants showed poor organogenesis but moderate callus induction. On IAA + BA, 2 mg/L IAA + 2 mg/L BA produced one shoot with two roots. On 2,4-D + BA, maximum callus was obtained at 0.5 mg/L 2,4-D + 1 mg/L BA. On 2,4-D + Kin, 2 mg/L 2,4-D with 0.5–2.5 mg/L Kin produced solid cream callus (Table 2).

Table 2 Results of petiole explant response

Hormone combination (mg/L)	Best response
IAA 2 + BA 2	1 shoot + 2 roots + green solid callus
NAA 0.5 (alone)	Dark brown semi-friable callus
2,4-D 0.5 + BA 1	Maximum callus (solid green)
2,4-D 2 + Kin 0.5–2.5	Solid cream callus

3.1.3. Nodal Explants

Nodal explants showed minimal callus but consistent single shoot regeneration. On IAA + BA, 0.5 mg/L IAA + 2.5 mg/L BA gave elongated shoots with solid green callus. On IAA + Kin, 2 mg/L IAA + 2.5 mg/L Kin produced a long shoot with numerous white roots. On 2,4-D, maximum callus was at 1 mg/L 2,4-D + 1.5 mg/L BA (Table 3).

Table 3 Results of nodal explant response

Hormone combination (mg/L)	Shoots	Roots	Callus
IAA 0.1 (alone)	1	15–20 thin	None
IAA 0.5 + BA 2.5	Elongated single	-	Solid green
IAA 2 + Kin 2.5	Single long	Numerous white	Dark brown
NAA 2 (alone)	None	-	Brown semi-friable
2,4-D 1 + BA 1.5	None	-	Maximum
2,4-D 2 + BA 2	None	5 white thin	Pale green-brown

3.1.4. Leaf Explants

Leaf explants showed the most diverse response. Maximum shoot regeneration (32 shoots/explant) was obtained on 0.1 mg/L IAA + 2.5 mg/L Kin, while 0.05 mg/L IAA + 2.5 mg/L Kin produced >50 shoot initials. On Kin alone (3 mg/L), 29 shoots/explant were produced. On 2,4-D alone, 2 mg/L induced maximum pale white solid callus (Table 4).

Table 4 Results of leaf explant response

Hormone combination (mg/L)	Best response
IAA 0.1 + Kin 2.5	32 shoots/explant
IAA 0.05 + Kin 2.5	>50 shoot initials
Kin 3 (alone)	29 shoots/explant
IAA 2 + BA 2	Solid dark green callus (entire surface)
NAA 1 + BA 2	Maximum black callus (adaxial side)
NAA 1 + Kin 2	Direct roots + organoids
2,4-D 2 (alone)	Maximum pale white solid callus

3.2. *In Vitro* Rooting

Shoots regenerated from leaf explants were transferred to rooting media. Optimum rooting (24 white roots of uniform length) was obtained on 0.5 mg/L NAA. On 2 mg/L IAA, 13 thick white roots with slight callus were produced (Table 5).

Table 5 Effect of auxins on *in vitro* rooting

Auxin (mg/L)	No. of roots	Root morphology	Callus
NAA 0.5	24	White, uniform	None
IAA 2	13	Thick white	Slight
IBA 1.5	1 main + 4–5 lateral	Thick main	None
IBA 1 + IAA 1.5	30–40 (small)	3–5 cm long	Basal

3.3. Hardening and Acclimatization

Rooted plantlets on 0.5 mg/L NAA were hardened in vermiculite with half-strength MS liquid medium. After two weeks, plantlets were transferred to soil. Plants reached 40 cm (10–12 leaves) after 45 days and 75 cm after 75 days. Flowering occurred after one year with 80% field survival.

3.4. Suspension Culture

Maximum packed cell volume (PCV) was obtained with 2 mg/L 2,4-D (0.756 mL/mL), followed by IAA (0.206) and NAA (0.177). No steroids were detected in cell pellets, but TLC of the medium revealed five fractions (Rf 0.104–0.594).

3.5. Biochemical Analysis

3.5.1. Total Carbohydrate and Protein

Maximum carbohydrate (10.623 mg/g FW) was in *in vitro* shoots, while minimum (6.320 mg/g) was in *in vivo* shoots at flowering. Maximum protein (12.654 mg/g) was in flowering *in vivo* shoots, and minimum (4.369 mg/g) in *in vitro* shoots (Table 6).

Table 6 Carbohydrate and protein content in different samples

Sample	Carbohydrate (mg/g FW)	Protein (mg/g FW)
Field-grown shoots (control)	10.215	9.364
In vitro shoots	10.623	4.369
In vivo shoots (5 months)	8.965	9.520
In vivo shoots (flowering)	6.320	12.654
Callus (2,4-D 2 mg/L)	9.025	8.321
Callus (2,4-D 1 + Kin 1.5)	8.965	9.125

3.5.2. Protein Profile (SDS-PAGE)

SDS-PAGE revealed seven bands in all samples except callus. A band at Rm 0.569 was unique to flowering *in vivo* shoots. A band at Rm 0.613 was absent in *in vitro* and *in vivo* shoots. Callus lacked the band at Rm 0.220 present in control.

3.6. Phytochemical Analysis

3.6.1. Steroid Yield

Control shoots contained the highest petroleum ether extractable residue (2.48%). Total steroid yield was similar in control and *in vitro* shoots (1 g each). Minimum steroid content (0.49 g) was obtained from callus (Table 7).

Table 7 Petroleum ether extract and steroid yield from different samples

Sample	Petroleum ether residue (%)	Total steroid yield (g)
Control shoot	2.48	1.00
In vitro shoot	<2.48	1.00
In vivo shoot (5 months)	<2.48	0.75
Control root	Low	0.50
Callus	Low	0.49

3.6.2. TLC Fractionation

Control and *in vitro* shoots contained seven steroid fractions (Rf 0.090–0.939). Fractions III and VI were absent in *in vivo* shoots. Callus from 1 mg/L 2,4-D + 1.5 mg/L Kin produced an eighth fraction (Rf 0.353). Suspension culture medium yielded five fractions (Table 8).

Table 8 Results of TLC steroid fractions from different samples

Sample	No. of fractions	Missing fractions	New fraction
Control shoot	7	-	-
In vitro shoot	7	-	-
In vivo shoot (5 months)	5	F-III, F-VI	-
In vivo shoot (flowering)	5	F-III, F-VI	-
Callus (2,4-D 1 + Kin 1.5)	8	-	Rf 0.353
Callus (2,4-D 2)	6	F-II	-
Suspension (medium)	5	F-II, F-VII	-

3.6.3. Identification of Steroids

Using specific spray reagents and co-chromatography with standards: solasodine (Rf 0.243) was identified with antimony trichloride (dark violet); β-sitosterol (Rf 0.315) with Libermann-Burchard (pale violet); diosgenin with Dragendorff's reagent (purple). Solasodine was absent in 5-month-old and flowering in vivo shoots. IR spectroscopy confirmed cholesterol (F-I), solasodine (F-III), β-sitosterol (F-IV), diosgenin (F-V), and betulin (F-VI).

3.6.4. Quantification of Major Steroids

Maximum steroid concentrations were in control and in vitro shoots; minimum in in vivo shoots. Solasodine content was 1.107 mg/g (control) and 1.007 mg/g *(in vitro*), but absent in in vivo shoots (Table 9).

Table 9 Quantification of major steroids (mg/g dry weight)

Steroid	Control shoot	*In vitro* shoot	*In vivo* shoot
Solasodine	1.107	1.007	Absent
β-sitosterol	1.272	1.102	0.986
Diosgenin	1.114	1.004	0.992

3.7. In Vitro Cytotoxicity

3.7.1. Aqueous Extract

At 400 µg, 50% cell death was observed for both control and *in vitro* shoot extracts. Root extracts caused 38–44% cell death at the same concentration (Table 10).

Table 10 Cytotoxicity of aqueous extracts at 400 µg (% cell death)

Sample	Shoot	Root
Control	50.00	38.56
In vitro	50.00	44.63
In vivo	26.55	34.54

3.7.2. Petroleum Ether Extract

At 100 µg, control shoot extract caused 91% cell death, followed by in vitro shoot from 3 mg/L Kin (83%) and callus extracts (73–76%) (Table 11).

Table 11 Cytotoxicity of petroleum ether extracts at 100 µg

Sample	Cell death (%)
Control shoot	91
In vitro shoot (Kin 3)	83
In vitro shoot (IAA 0.1 + Kin 2.5)	~80
Callus (2,4-D 1 + Kin 1.5)	76
Callus (2,4-D 2)	73

3.7.3. Total Steroid Fractions

At 80 µg, total steroid fractions from control shoots and in vitro shoots (Kin 3) caused 100% cell death. Suspension culture also showed 100% death at 80 µg. *In vivo* root showed the least cytotoxicity (Table 12).

Table 12 Cytotoxicity of total steroid fractions

Sample	Concentration (µg)	Cell death (%)
Control shoot	80	100
In vitro shoot (Kin 3)	80	100
Suspension culture	80	100
Callus (2,4-D 1 + Kin 1.5)	100	100
In vivo root	100	Least

3.7.4. Fractionated Steroids (CC_{50} Values)

The concentration required for 50% cell death (CC_{50}) varied among fractions and samples. Fraction V (diosgenin) and Fraction VI (betulin) showed the lowest CC_{50} values, indicating higher potency (Table 13).F

Table 13 CC_{50} values (µg) of selected steroid fractions from different samples

Sample	Fraction	CC_{50} (µg)
Control shoot	F-V	22.5
Control shoot	F-III (solasodine)	55.0
In vitro shoot	F-VI	21.0
In vitro shoot	F-V	23.0
In vivo shoot	F-V	61.0
Control root	F-I	39.5
Callus	F-IV	37.5
Callus	F-VI	39.0

4. Discussion

The present study successfully established a high-frequency regeneration protocol for *Solanum trilobatum* L., demonstrating that explant selection and hormonal balance critically determine morphogenic outcomes. Leaf explants showed superior shoot regeneration (32 shoots/explant on 0.1 mg/L IAA + 2.5 mg/L Kin) compared to shoot tips, nodes, and petioles. This differential response aligns with previous reports in *Solanum* species, where leaf explants produced the highest number of shoots on medium containing low auxin and high cytokinin concentrations (Govindarajan and Chinnachamy, 2015; Saritha et al., 2007). The effectiveness of this combination is consistent with the classical cytokinin-driven shoot organogenesis model (Skoog and Miller, 1957). Nodal explants produced only single shoots, while petiole explants showed poor organogenesis but moderate callus induction, similar to findings by Kumar et al. (2010) and Ramachandra Rao and Ravishankar (2002).

The induction of friable callus on 2 mg/L 2,4-D alone and on 1 mg/L 2,4-D + 1.5 mg/L Kin from leaf explants is significant for establishing suspension cultures. Maximum packed cell volume (0.756 mL/mL) was obtained with 2 mg/L 2,4-D, comparable to reports in *Solanum nigrum* (Rani and Rana, 2015). The pale yellow friable callus obtained is desirable for biomass production and secondary metabolite extraction (Patel and Rao, 2014).

Biochemical analysis revealed maximum carbohydrate content in *in vitro* shoots (10.623 mg/g FW), supporting the observation that sucrose-supplemented MS medium enhances photosynthetic assimilate accumulation (Anirudhan and Nair, 2012). Protein content peaked in flowering *in vivo* shoots (12.654 mg/g FW) and was minimal in *in vitro* shoots (4.369 mg/g FW), reflecting developmental stage-specific expression (Shilpa et al., 2015). SDS-PAGE revealed seven bands in all samples except callus, with a unique band at Rm 0.569 only in flowering shoots, suggesting genetic fidelity of regenerated plants (Shilpa et al., 2014).

Phytochemical analysis showed that control and *in vitro* shoots contained seven steroid fractions, while *in vivo* shoots lost fractions F-III and F-VI. Callus produced an additional eighth fraction (Rf 0.353), indicating stress-induced novel metabolite production (Murthy et al., 2014). Solasodine was identified at Rf 0.243 using antimony trichloride, β-sitosterol at Rf 0.315 using Libermann-Burchard reagent, and diosgenin using Dragendorff's reagent, consistent with Mohanan et al. (1998) and Emke and Eilert (1986). Quantification revealed solasodine content of 1.107 mg/g in control shoots and 1.007 mg/g in *in vitro* shoots, but complete absence in *in vivo* shoots. This striking loss during acclimatization contrasts with some reports (Shilpa et al., 2015) but supports the finding that manipulated *in vitro* conditions can enhance solasodine yield up to 35.97 mg/g DW (Anirudhan and Nair, 2012).

Cytotoxicity studies using DLA cells demonstrated that both control and *in vitro* shoot aqueous extracts caused 50% cell death at 400 µg, while *in vivo* shoots caused only 26.55%. Total steroid fractions from control and *in vitro* shoots (Kin 3 mg/L) caused 100% cell death at 80 µg, comparable to suspension culture. CC_{50} values showed F-V (diosgenin, 22.5 µg) and F-VI (betulin, 21.0 µg) as most potent. These findings align with Mohanan et al. (1998), who reported LD_{50} values of 7.0 µg (L929) and 5.8 µg (Vero) for petroleum ether extracts, and with Muthu and Michael (2012), who demonstrated potent activity against DLA and EAC cell lines.

Hardening and acclimatization yielded 80% field survival, comparable to previous reports (Josekutty and Prathapasenan, 2012; Sridhar and Naidu, 2011). Regenerated plants flowered after one year, confirming developmental

competence. The antidiabetic (Rajasulochana et al., 2016) and hepatoprotective (Shahjahan et al., 2005) equivalence of micropropagated *S. trilobatum* further supports the therapeutic validity of *in vitro*-derived materials.

In conclusion, leaf explants with 0.1 mg/L IAA + 2.5 mg/L Kin are optimal for high-frequency regeneration of *S. trilobatum*. While *in vitro* shoots maintain solasodine levels and superior cytotoxic activity, the loss of solasodine during field acclimatization suggests that direct harvesting of *in vitro* shoots or suspension cultures may be preferable for steroidal alkaloid production. The cytotoxic equivalence of *in vitro*-derived materials validates micropropagation as a reliable strategy for producing therapeutically potent biomass (Mohanan et al., 1998; Sundaram et al., 2019).

5. Conclusion

The present study established an efficient and reproducible micropropagation protocol for *Solanum trilobatum* L. using leaf explants cultured on MS medium supplemented with 0.1 mg/L IAA and 2.5 mg/L Kin, which produced the highest number of shoots (32 shoots/explant). Among the various explants tested, leaf explants showed superior regenerative competence compared to shoot tips, nodes, and petioles. Friable callus suitable for suspension cultures was successfully induced on 2 mg/L 2,4-D alone and on 1 mg/L 2,4-D with 1.5 mg/L Kin. Biochemical analysis revealed that *in vitro* shoots accumulated higher carbohydrate content than field-grown controls, while protein content peaked in flowering *in vivo* shoots. SDS-PAGE confirmed the genetic fidelity of regenerated plants.

Phytochemical analysis demonstrated that both control and *in vitro* shoots contained seven steroid fractions, including solasodine (1.107 and 1.007 mg/g respectively), which was completely absent in *in vivo* shoots after field transfer. This finding has significant implications for commercial solasodine production, suggesting that direct harvesting of *in vitro* shoots or suspension cultures may be preferable to field cultivation for steroidal alkaloid extraction. Cytotoxicity studies against DLA cell lines confirmed that *in vitro*-derived materials (aqueous and steroid extracts) showed equivalent or superior anticancer activity compared to field-grown controls, with total steroid fractions causing 100% cell death at 80 µg. The successful hardening and acclimatization of regenerated plantlets with 80% field survival further supports the practical applicability of this protocol for conservation and large-scale propagation. Overall, this study validates plant tissue culture as a reliable strategy for producing therapeutically potent and chemically consistent biomass of *S. trilobatum*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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