Extraction of Total phenolics from callus cell suspension cultures of Celastros Paniculatus wild

Author: Sandipkumar Chandulal Patel
(Research Scholar)
Prof.. (Dr.) K.S.Dahiya
Botany Department, Madhav University,
Abu Road (Rajasthan)

Abstract

Celastrus paniculatus Willd., also known as Jyotishmati or Malkangani, is a highly valued medicinal herb in Ayurvedic medicine, famous for its neuroprotective and memory-boosting effects. This research outlines an optimised method for generating callus cultures, developing cell suspensions, and extracting phenolic compounds from C. paniculatus. Leaf explants cultured on MS medium with 2,4-D (1.0 mg/L) and BAP (0.5 mg/L) achieved 93% callus induction within 21 days. Cell suspension cultures in liquid MS medium produced maximum phenolics (14.2 ± 0.8 mg GAE/g DW) at 14 days. Spectrophotometric (Folin-Ciocalteu) and HPLC-DAD analyses identified notable levels of gallic acid, chlorogenic acid, and quercetin derivatives. The optimised extraction using 70% ethanol at 40°C for 30 minutes resulted in 28% higher phenolic yields than traditional methods. This biotechnological approach provides a sustainable alternative to wild harvesting and boosts phenolic compound production.

Keywords: Celastrus paniculatus, callus culture, cell suspension, phenolic compounds, HPLC, antioxidant activity

1. Introduction

Celastrus paniculatus Willd. (Celastraceae family) has been extensively used in traditional Indian medicine for centuries, primarily for its remarkable neuropharmacological properties. The plant's therapeutic potential is attributed to its rich content of secondary metabolites, particularly phenolic compounds, which exhibit potent antioxidant, anti-inflammatory, and neuroprotective activities. Recent pharmacological studies have demonstrated that phenolic extracts from C. paniculatus exhibit significant free radical scavenging activity, with IC50 values comparable to those of standard antioxidants, such as ascorbic acid.

The growing demand for these bioactive compounds, coupled with the plant's vulnerable conservation status (listed as Vulnerable by IUCN in 2022) due to overharvesting and habitat destruction, has created an urgent need for alternative production methods. Traditional extraction from wild plants presents several limitations, including seasonal variability in metabolite content, low yields (typically 0.8-1.2% of the dry weight), and ecological concerns regarding sustainable harvesting practices.

Plant cell culture technology offers a promising solution to these challenges. Callus cell suspension cultures provide several distinct advantages over conventional plant material: (1) Controlled, year-round production independent of seasonal variations; (2) Potential for higher metabolite yields through optimization of culture conditions; (3) Reduced environmental impact by minimizing wild harvesting; and (4) Consistent production of standardized phytochemical extracts. Previous studies have demonstrated that in vitro cultures of various medicinal plants can produce 3-5 times higher concentrations of secondary metabolites compared to their wild counterparts.

This study aims to:

(1) Develop an optimised protocol for callus induction and cell suspension culture establishment from C. paniculatus;

(2) Determine the optimal conditions for phenolic compound production in suspension cultures;

(3) Compare different extraction methods for maximum phenolic yield; and

(4) Characterise the phenolic profile using advanced analytical techniques.

2. Materials and Methods

2.1. Plant Material and Sterilisation

Young leaves were sourced from verified C. paniculatus plants kept in the institutional greenhouse (Herbarium voucher no. BSI/SRC/CP-2023-01). The explants were carefully sterilised: first washed with running tap water for 15 minutes, then treated with 2% (v/v) Teepol solution for 5 minutes, immersed in 70% ethanol for 45 seconds, and surface sterilised with 0.1% (w/v) mercuric chloride solution for 3 minutes. After sterilisation, the explants were rinsed three times with sterile distilled water in aseptic conditions.

2.2. Callus Induction and Maintenance

Sterilised leaf explants, approximately 1 cm^2 in size, were placed on Murashige and Skoog (MS) medium containing different combinations and levels of plant growth regulators (PGRs). The media formulations included: (1) MS basal medium (control); (2) MS with 2,4-D at 0.5-2.0 mg/L; (3) MS with 1.0 mg/L 2,4-D plus BAP at 0.1-1.0 mg/L; and (4) MS with NAA at 0.5-2.0 mg/L plus kinetin at 0.1-1.0 mg/L. The pH of all media was adjusted to 5.8 before autoclaving at 121°C for 15 minutes. Cultures were maintained at 25±2°C under a 16/8-hour light/dark cycle with a light intensity of 50 $\mu mol\ m^{-2}\ s^{-1}$ provided by cool-white fluorescent lamps. The frequency of callus induction, growth rate, and morphology were assessed every 7 days over 4 weeks.

2.3. Establishment of Cell Suspension Cultures

A friable callus weighing about 5 g (fresh weight) was placed into 250 mL Erlenmeyer flasks containing 100 mL of liquid MS medium, with the optimal PGR combination identified from callus induction experiments. These cultures were maintained on an orbital shaker at 110 rpm in continuous darkness at 25 °C ± 1 °C. Cell growth was tracked by measuring packed cell volume (PCV) and dry weight every 3 days over the 21-day culture period. To measure PCV, 10 mL samples of the cell suspension were transferred into graduated centrifuge tubes and centrifuged at 200 × g for 5 minutes.

2.4. Extraction of Phenolic Compounds

The extraction of phenolic compounds was systematically optimized by evaluating various parameters: (1) Solvent type (methanol, ethanol, acetone); (2) Solvent concentration (50%, 70%, 90%); (3) Extraction time (15, 30, 60 minutes); (4) Temperature (25, 40, 60°C); and (5) Solvent-to-biomass ratio (5:1, 10:1, 15:1 v/w). The

optimised protocol employed 70% ethanol with 0.1% HCl, using a solvent-to-biomass ratio of 10:1 (v/w) at 40°C for 30 minutes with continuous shaking at 150 rpm. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 40°C using a rotary evaporator.

3. Results and Discussion

3.1. Callus Induction and Growth Kinetics

The callus induction experiments revealed significant variation in response depending on explant type and PGR combinations. Leaf explants showed superior callus formation (93% induction frequency) compared to stem segments (78%) or petioles (65%). Among the various PGR combinations tested, MS medium supplemented with 2,4-D (1.0 mg/L) and BAP (0.5 mg/L) proved to be the most effective, producing friable, yellowish callus within 21 days (Figure 1). This combination not only promoted high callus induction rates but also maintained the desired friable morphology essential for establishing suspension cultures. Microscopic examination revealed that the most productive callus lines consisted of small, densely cytoplasmic cells with prominent nuclei, characteristic of metabolically active tissues.

3.2. Cell Suspension Culture Development

The cell suspension cultures exhibited a typical sigmoidal growth pattern (see Figure 2), consisting of three phases: (1) Lag phase from days 0 to 3; (2) Exponential growth phase from days 4 to 14; and (3) Stationary phase from days 15 to 21. The highest biomass accumulation was 12.3 ± 0.6 g/L DW on day 14, aligning with the late exponential phase. Phenolic production displayed a clear pattern, reaching its peak (14.2 ± 0.8 mg GAE/g DW) at the same late exponential phase (day 14) and then gradually decreasing as the cultures transitioned into the stationary phase. This pattern suggests that phenolic biosynthesis in C. paniculatus is closely linked to primary metabolic activities during active cell growth.

4. Conclusion

This study effectively developed a streamlined protocol for producing and extracting phenolic compounds from C. paniculatus callus suspension cultures. The optimised approach shows great promise for sustainable phytochemical production, presenting a feasible alternative to wild harvesting of this vulnerable medicinal plant. Future work should aim to scale up the process using bioreactors and explore elicitation techniques

to boost phenolic yields. This protocol not only supports conservation efforts but also offers a reliable platform for producing standardised extracts for pharmaceutical use.

References

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