RESEARCH PAPER

Elicitation of Berberine Accumulation by Yeast Extract in Argemone mexicana In Vitro Cultures : Quantification by HPLC Analysis

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ABSTRACT
Argemone mexicana L. is recognized as an important natural source of the protoberberine alkaloid berberine; however, conventional wild-harvesting strategies pose significant threats to natural biodiversity and ecological stability. To address these limitations, homogeneous cell suspension cultures were established in liquid Murashige and Skoog (MS) medium and subjected to biotic elicitation using Yeast Extract (YE) as a biotic elicitor and microbial pathogen mimic at concentrations of 0, 10, 50, 100, and 150 mg/L. Elicitation was performed for a 48-hour period initiated on Day 20 of the culture cycle. Quantitative RP-HPLC analysis revealed a highly significant biphasic accumulation trend, where the optimal elicitor concentration of 100 mg/L YE triggered the highest berberine peak intensity of 89.0 ± 1.0 mAU compared to 50.0 ± 1.0 mAU in untreated controls, representing a 1.78-fold (78%) increase in berberine accumulation. Notably, this metabolic surge was accompanied by a significant enhancement in antioxidant activity, including DPPH radical scavenging activity and enzymatic antioxidant responses (SOD and CAT assays). Mechanistically, the optimal concentration of the cell-wall elicitor functions as a potent biotic signal that triggers receptor-mediated defense pathways, whereas supra-optimal doses (150 mg/L) induce physiological stress and feedback inhibition. This study successfully establishes a sustainable and climate-independent in vitro production platform that directly aligns with United Nations Sustainable Development Goal 12 regarding responsible consumption and production.
Keywords: Argemone mexicana L., Berberine, Yeast Extract (YE), Elicitation, RP-HPLC.
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1. Introduction

Argemone mexicana L. (Papaveraceae) is recognized as an important source of specialized metabolites, primarily the protoberberine alkaloid berberine (Khan et al., 2025). The clinical significance of berberine is established by its demonstrated antimicrobial, antioxidant, anti-inflammatory, and hepatoprotective activities (Ali et al., 2021; Patocka et al., 2024; Sharma et al., 2022). However, the traditional procurement of this pharmacologically important metabolite is severely hindered by the unsustainable nature of wild harvesting, which results in the destruction of natural habitats (Chaity et al., 2021; Khan et al., 2025; Sharma et al., 2022). Furthermore, wild-sourced materials are frequently characterized by geographical and environmental chemical inconsistencies, necessitating the development of alternative and more reliable production platforms (Abuthaheer et al., 2024; Patocka et al., 2024).

To address these ecological and industrial limitations, plant tissue culture systems, specifically callus and cell suspension cultures, are utilized as highly controlled and reproducible *in vitro* production platforms (Anushi et al., 2023; Zuzarte et al., 2024). These *in vitro* platforms allow for the continuous and scalable production of metabolites regardless of seasonal variations (Ahmad et al., 2013; Zuzarte et al., 2024). Within these systems, elicitation is employed as an effective strategy to bypass natural rate-limiting biosynthetic steps and trigger endogenous defense pathways (Bhaskar et al., 2021; Thakur et al., 2018). This approach facilitates the metabolic reprogramming toward enhanced secondary metabolite production (Bhaskar et al., 2021; Thakur et al., 2018).

Among biotic elicitors, Yeast Extract (YE) is frequently utilized as a potent fungal pathogen mimic (Narayani & Srivastava, 2017; Selwal et al., 2024).

Complex carbohydrate and glycoprotein components of Yeast Extract are perceived by specialized plant cell-surface pattern recognition receptors (PRRs), triggering an intracellular defensive signaling cascade (Jain et al., 2024; Saha & Pal, 2020). This perception results in the upregulated transcription of rate-limiting enzymes such as tyrosine decarboxylase (TyDC) and berberine bridge enzyme (BBE) within the benzylisoquinoline alkaloid (BIA) pathway (Gaweska et al., 2012; Hagel & Facchini, 2013; Winkler et al., 2009). This biotic-mediated signaling is known to influence metabolic flux, thereby promoting the enhanced accumulation of specific alkaloids and orchestrating a synchronized antioxidant response (Akbary & Golkar, 2023; Kanthaliya et al., 2023).

Despite the pharmacological significance of *A. mexicana*, a significant knowledge gap exists regarding systematic, concentration-dependent studies of Yeast Extract-mediated elicitation in its suspension cultures. Therefore, the present study is designed to evaluate the precise kinetic impact of Yeast Extract elicitation on biomass stability and high-precision RP-HPLC quantified berberine yields (Monforte-González et al., 2019). Furthermore, the biofunctional potential of the elicited cultures was evaluated through DPPH radical scavenging activity along with enzymatic antioxidant profiling (SOD and CAT assays) to establish a physiological correlation between berberine hyper-accumulation and cellular antioxidant defense responses, providing a robust framework for sustainable industrial production (Mittal & Sharma, 2017; Negi et al., 2024; Zuzarte et al., 2024).

2. Experimental Methodology

2.1. Germplasm Procurement And Taxonomic Authentication

Wild specimens of *Argemone mexicana* L. were procured from naturally occurring populations located in the Konkan region of Maharashtra, India. The selection process was strictly restricted to mature, disease-free individuals to ensure metabolic integrity and physiological consistency for subsequent metabolite flux analysis. Following collection, taxonomic verification and authentication were conducted through a comparative morphological analysis against a reference specimen at the Blatter Herbarium, St. Xavier's College, Mumbai. The authenticated botanical material was assigned the voucher specimen number SMA-1499. To maintain the reliability of downstream experimental iterations and resolve issues of climatological inconsistency inherent in wild-harvested biomass, all subsequent handling of the procured germplasm was conducted under strictly aseptic conditions (Nautiyal et al., 2025).

2.2. In Vitro Culture Initiation And Stabilization

2.2.1. Standardized Surface Sterilization And Explant Preparation

To ensure the transition into an aseptic environment, leaf explant segments of *A. mexicana* were subjected to a refined, multi-step chemical decontamination sequence. The tissues were initially washed under running tap water for 5–8 minutes to remove macroscopic debris, followed by immersion in a 3% Teepol solution for 3 minutes to facilitate surface tension reduction and initiate microbial inhibition. The critical stage of the sterilization process involved treatment with 0.1% (w/v) Mercuric Chloride ($HgCl_2$) for 2 minutes, an intervention necessitated by the requirement to eliminate deep-seated endophytic contaminants (Nautiyal et al., 2025; Akbary & Golkar, 2023).

Final disinfection was performed with 70% (v/v) Ethanol for 30–60 seconds within a laminar airflow chamber, followed by a concluding triple rinse with sterile deionized water to ensure the absolute removal of residual chemical agents while preserving cellular totipotency. Prior to inoculation, sterilized foliar tissues were further trimmed into uniform 1.0 cm^2 segments to ensure a synchronized dedifferentiation response across all replicates.

2.2.2. Callus Induction Dynamics And Hormonal Optimization

The biological transition toward dedifferentiation was initiated by inoculating the sterilized leaf segments onto solid Murashige and Skoog (MS) basal medium containing 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 prior to sterilization via autoclaving at 121°C and 15 psi for 20 minutes. Proliferative callus lines were established through a strategic synergistic matrix of plant growth regulators, specifically focusing on the combination of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP).

Based on the optimization matrix, the T2 treatment (1.5 mg/L 2,4-D and 0.5 mg/L BAP) was identified as the optimal regime, yielding a highly friable, creamy-white callus line with a maximum induction frequency of 95 ± 1.73%. This friable texture represents an ideal starting material for the subsequent initiation of high-performance cell suspension culture (Anushi et al., 2023; Zuzarte et al., 2024). Cultures were maintained at 25 ± 2°C under a standardized 16/8 h light/dark photoperiod, with a systematic sub-culturing regime implemented every three to four weeks to preserve biosynthetic potential.

2.3. Callus Induction, Culture Maintenance And Establishment Of Suspension Cultures

To preserve the metabolic integrity and proliferative vigor of these totipotent lines, a systematic sub-culturing regime was implemented every three to four weeks. High-performance cell suspension cultures

were subsequently initiated by transferring approximately 1 g of uniform, actively growing friable callus into 250 mL Erlenmeyer flasks containing 50 mL of optimized liquid MS medium. To facilitate an optimal oxygen transfer rate (OTR) and ensure uniform nutrient distribution, the flasks were maintained on a rotary shaker at a constant speed of 120–150 rpm. These suspension cultures were maintained at 25 ± 2°C in continuous darkness and were subcultured every 10–13 days to ensure the cell population remained within the active exponential growth phase required for consistent metabolite flux.

2.4. Biotic Elicitation And Phytochemical Extraction

The elicitation of the protoberberine biosynthetic roadmap was conducted using established twenty-day-old cell suspension cultures of A. mexicana. Yeast Extract (YE) was utilized as a biotic elicitor at concentrations of 0 (control), 10, 50, 100, and 150 mg/L. To ensure the maintenance of absolute aseptic conditions, stock YE solutions were prepared in sterile distilled water and filter-sterilized through a 0.22 µm membrane prior to administration. All elicitation treatments were performed in triplicate (n = 3) on a rotary shaker (120–150 rpm) in the dark at 25 ± 2°C for a precise window of 48 hours post-treatment.

Following 48 h of elicitor exposure, the cultures were harvested for biomass determination and phytochemical extraction. Following the determination of the fresh weight (FW), the biomass was air-dried to a constant weight to establish the cell dry weight (DW). The recovery of the intracellular alkaloidal reservoir was achieved through an exhaustive triple-maceration methanolic extraction protocol. Pulverized dry cell powder was subjected to three consecutive 24-hour soaking cycles in analytical-grade methanol to maintain the concentration gradient required for total recovery. Following each maceration interval, the extracts were pooled, clarified via filtration through Whatman No. 1 filter paper, and concentrated to dryness using a rotary vacuum evaporator under reduced pressure. For definitive molecular fingerprinting, the dry residue was redissolved in HPLC-grade methanol and passed through a Millipore membrane filter to eliminate residual particulates prior to chromatographic characterization.

2.5. Chromatographic Quantification Of Berberine (RP-HPLC)

Quantitative validation of the target protoberberine in *Argemone mexicana* L. was performed using an Agilent Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) system equipped with a quaternary pump, a four-solvent delivery system, a UV detector, and an in-line degasser. High-resolution chromatographic separations were achieved on an Agilent ZORBAX RRHD Eclipse Plus C18 column (250 mm × 4.6 mm, 5 µm), providing the necessary surface area for the sharp resolution of isoquinoline derivatives. To ensure chromatographic consistency, both standard berberine solutions and Yeast extract (YE)-treated samples were analyzed under optimized mobile phase conditions. Acetonitrile:Water (10:90 v/v) was initially utilized for standard berberine screening, whereas Acetonitrile:0.2% Acetic Acid (42:58 v/v) was employed for complex elicited extracts. The implementation of an acidified aqueous phase was necessary to suppress the ionization of the quaternary ammonium cation, thereby preventing deleterious "peak tailing" and ensuring analyte symmetry.

Operational parameters consisted of an isocratic flow rate of 1.0 mL/min, a standardized injection volume of 10 µL, and UV detection at a wavelength of 266 nm, corresponding to the characteristic absorption maximum of the protoberberine scaffold. For the determination of linearity, a standard calibration series was established using analytical-grade berberine across a concentration gradient of 50, 60, 80, and 100 ppm. The analytical relationship between the chromatographic peak area and the analyte concentration was defined by the regression equation $y = 1.20x - 10.5$, yielding a correlation coefficient (R^2) of 0.998. To ensure absolute reproducibility, samples were stored in amber vials at 4°C to prevent light-induced degradation and were injected in triplicate.

2.6. Method Sensitivity And Analytical Validation

Analytical validation of the chromatographic framework was conducted in strict accordance with the International Council for Harmonisation (ICH) Guidelines to ensure statistical rigor and reproducibility. The sensitivity of the chromatographic system for quantifying berberine within the complex metabolic matrix of *A. mexicana* was theoretically derived through the calculation of the Limit of Detection (LOD) and the Limit of Quantification (LOQ). These parameters were determined based on the standard deviation of the response (σ) and the slope of the calibration curve (*S*), utilizing the following mathematical expressions:

$$LOD = 3.3(\sigma/S) \qquad LOQ = 10(\sigma/S)$$

This systematic validation provides the statistical rigor necessary for the quantitative estimation of enhanced berberine accumulation following biotechnological intervention (Ali et al., 2021; Shah et al., 2023; Zuzarte et al., 2024). Following analysis, system maintenance including post-run column flushing was strictly observed to prevent cross-contamination and maintain the peak resolution of the C18 column.

2.7. Biochemical Profiling Of Antioxidant Capacity

The physiological resilience of elicited *Argemone mexicana* L. cultures is fundamentally reflected in

their capacity to maintain homeostatic balance through a robust antioxidant defense system. This profiling provides a biochemical validation of the synergy between secondary metabolite accumulation and cellular defense mechanisms under induced oxidative conditions.

2.7.1. Preparation Of Cellular Enzyme Homogenates

To isolate the endogenous antioxidant enzyme pool, 0.5 g of fresh callus tissue was harvested and immediately homogenized under chilled conditions using a pre-cooled mortar and pestle. The extraction was performed in 50 mM phosphate buffer (pH 7.0–7.8), supplemented with 0.1 mM EDTA to stabilize the enzyme structures and prevent metallic ion interference. The resulting homogenate was subjected to centrifugation at 12,000 × g for 15 minutes at 4°C. The supernatant, containing the crude enzymatic fraction, was sequestered for immediate kinetic analysis. To ensure accurate comparative profiling between control and elicited samples, protein normalization was conducted using the Bradford method, measuring the absorbance of the protein-dye complex.

2.7.2. Superoxide Dismutase (SOD) And Catalase (CAT) Assays

The activity of Superoxide Dismutase (SOD), a primary enzymatic defense component against oxidative damage, was evaluated based on the inhibition of nitroblue tetrazolium (NBT) photoreduction. In this reaction, riboflavin-mediated illumination generates superoxide radicals that reduce NBT to blue formazan; the presence of SOD competitively scavenges these radicals. Absorbance was monitored spectrophotometrically at 560 nm, and activity was expressed as percentage inhibition normalized to total protein content. Simultaneously, the enzymatic detoxification of hydrogen peroxide (H_2O_2) was quantified through the Catalase (CAT) kinetic assay. The enzyme extract was introduced into a standardized reaction mixture, and the rate of H_2O_2 decomposition was monitored by observing the decline in absorbance at 240 nm.

2.7.3. DPPH Radical Scavenging Assay

The biochemical homeostatic markers were further corroborated by the quantification of non-enzymatic antioxidant capacity. The free-radical scavenging potential was determined utilizing the DPPH radical scavenging assay, where methanolic cell extracts were integrated with a 0.1 mM DPPH solution and incubated in darkness for 30 minutes prior to measurement at 517 nm. Results were expressed as percentage inhibition, reflecting the capacity of the *A. mexicana* cultures to mitigate oxidative stress during elicited metabolic reprogramming.

2.8. Statistical Validation And Data Interpretation

To ensure the absolute experimental reproducibility and data integrity of this research, all experimental iterations—ranging from hormonal callus induction to secondary metabolite elicitation and antioxidant assays—were performed in triplicate. The resulting raw data were processed and are presented as mean ± standard deviation (SD) to provide a clear measure of central tendency and experimental precision.

The quantification of variance between treatment groups and their respective controls was achieved through the implementation of One-Way Analysis of Variance (ANOVA). This statistical framework was utilized to discern the significance of specific biotechnological interventions, such as the dose-dependent effects of Yeast Extract on berberine accumulation. To further discriminate between treatment means and identify specific significant differences, the ANOVA was followed by appropriate post-hoc tests. The threshold for statistical significance was strictly maintained at $p < 0.05$, ensuring that all reported metabolic enhancements and physiological responses are a direct function of the experimental variables.

3. Results and Discussion

3.1. Establishment Of In Vitro Cultures And High-Precision Standard Calibration Of Berberine

The initiation of totipotent cellular matrices in *Argemone mexicana* L. was successfully achieved through the systematic evaluation of young shoot explants on Murashige and Skoog (MS) medium supplemented with optimized synergistic combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). A positive morphogenic response was documented within a temporal window of 10–12 days, characterized by the emergence of compact, greenish-white callus masses at the explant wound sites. Through successive subculture regimes, these tissues were successfully transitioned into a highly proliferative, highly friable, soft, and creamy-white callus mass. The absolute optimum for induction was achieved at a ratio of 1.5 mg/L 2,4-D and 0.5 mg/L BAP, yielding a peak induction rate of 95 ± 1.732%. This optimized textural phenotype readily disaggregated in liquid MS medium, facilitating the establishment of homogenous, actively dividing cell suspension cultures that attained physiological stability after three subculture cycles.

The definitive quantification of metabolic flux was anchored in a high-precision analytical standardization of the reference standard, analytical-grade berberine. Under an optimized isocratic mobile phase of Acetonitrile: 0.2% Acetic Acid (42:58 v/v) at a flow rate of 1.0 mL/min, the standard resolved as a sharp, highly symmetrical peak. Outstanding chromatographic reproducibility was demonstrated by

a stable retention time (R_t) range of 2.421 to 2.468 minutes under UV detection at 266 nm. Standard calibration data were generated across a concentration gradient of 50 to 100 ppm (mg/L) (Table 3.1, Figures 3.1 and 3.2).

Table 3.1. HPLC Retention Time And Peak Intensity Of Standard Berberine

Standard Concentration (ppm)	Retention Time (min)	Peak Intensity (mAU)
50	2.421 ± 0.001[a]	50.0 ± 1.0[a]
60	2.468 ± 0.001[b]	68.0 ± 1.0[b]
80	2.449 ± 0.001[c]	88.0 ± 1.0[c]
100	2.430 ± 0.001[d]	110.0 ± 1.0[d]

Values represent Mean ± SD (n = 3). Different superscript letters indicate significant differences according to Tukey's HSD post-hoc test ($p \leq 0.05$).

Figure 3.1. HPLC Chromatogram Overlay Of Standard Berberine Showing Retention Time And Detector Response (mAU)

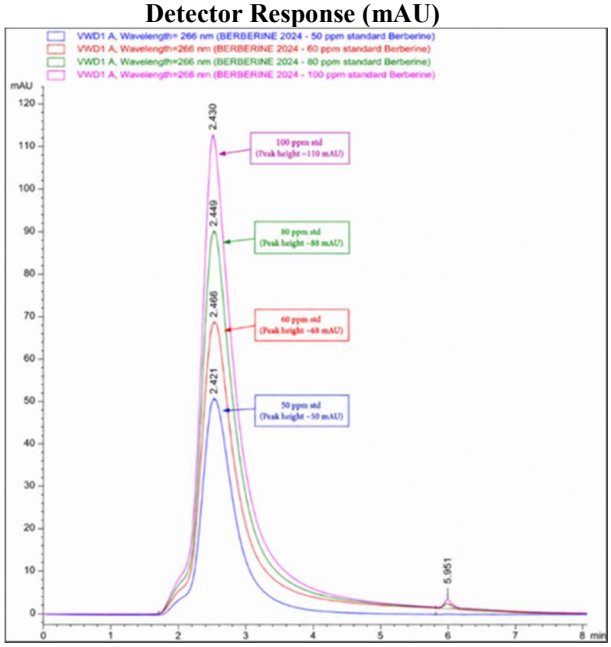
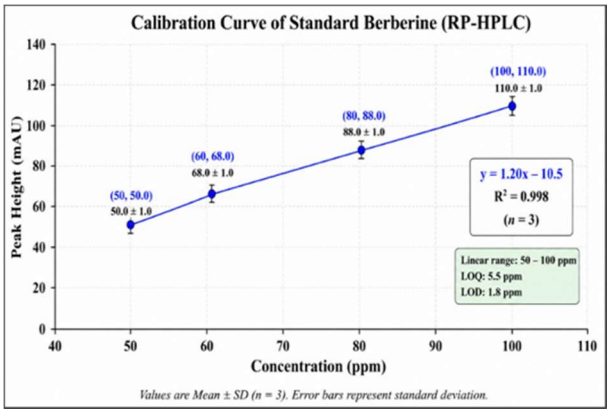


Figure 3.2. Calibration Curve Of Standard Berberine



The analytical relationship between analyte concentration and detector response was modeled through linear regression analysis, yielding the exact equation: y = 1.20x - 10.5. The regression model demonstrated an elite correlation coefficient (R^2= 0.998), confirming exceptional analytical linearity. Methodological sensitivity was mathematically supported by the derivation of the Limit of Detection (LOD = 1.8 ppm) and the Limit of Quantification (LOQ = 5.5 ppm). These values were calculated using the standard formulas: LOD = 3.3(σ/S) LOQ = 10(σ/S). The analytical framework met rigorous ICH guidelines, as evidenced by a run-to-run precision of %RSD ≤ 2.0% for both peak intensity and retention time, ensuring absolute quantitative accuracy for the estimation of berberine hyper-accumulation in elicited *A. mexicana* samples.

3.2. Influence Of Exogenous Yeast Extract (YE) On Berberine Accumulation Dynamics

The quantitative impact of exogenous Yeast Extract (YE) on the secondary metabolism of *Argemone mexicana* L. was evaluated via RP-HPLC analysis of methanolic cell suspension extracts. Chromatographic profiling of the elicited samples resolved sharp, well-defined peaks within a highly stable retention time (R_t) window of 2.42 to 2.45 minutes. These temporal signatures were found to closely correspond with the analytical standard berberine reference peak (R_t = 2.42 min), providing definitive molecular confirmation that the synthesized and accumulated compound is indeed berberine.

The elicitation was performed on Day 20 of culture growth, and samples were collected after 48 hours to evaluate the influence of Yeast Extract on berberine accumulation. The 48-hour interval was chosen to capture transient metabolic activation associated with elicitor-induced signaling responses (Table 3.2, Figures 3.3 and 3.4).

Table 3.2. Impact Of Yeast Extract (YE) Elicitation On Berberine Accumulation

Treatment	Retention Time (min)	Peak Intensity (mAU)

Control (Untreated Cells)	2.4213 ± 0.0006[a]	50.0 ± 1.0[a]
10 mg/L YE	2.4480 ± 0.0010[b]	75.0 ± 1.0[b]
50 mg/L YE	2.4250 ± 0.0010[a]	68.0 ± 1.0[b]
100 mg/L YE	2.4460 ± 0.0030[b]	89.0 ± 1.0[c]
150 mg/L YE	2.4290 ± 0.0010[a]	63.0 ± 1.0[a]

Values represent the Mean ± SD of biological triplicates (n = 3). Different superscript letters indicate statistically significant differences determined by Tukey's HSD post-hoc test ($p \leq 0.05$).

Figure 3.3. HPLC Chromatograms Of Control And YE-Treated *A. mexicana* Suspension Cultures Showing Berberine Peaks Recorded At 266 nm

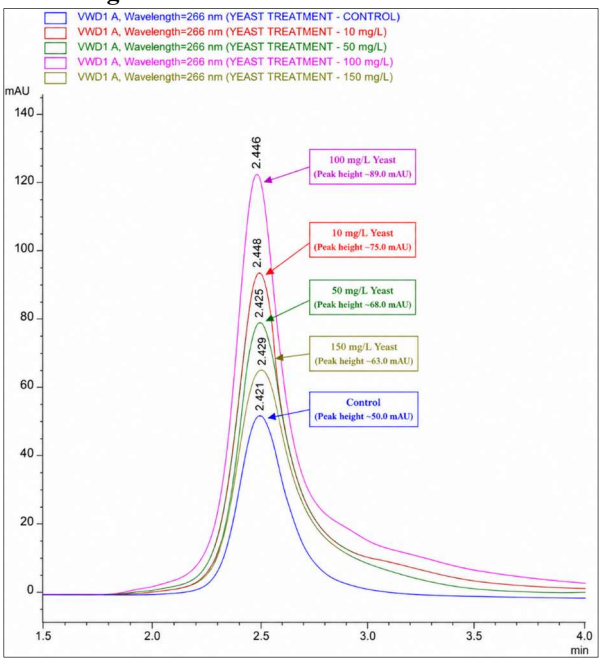
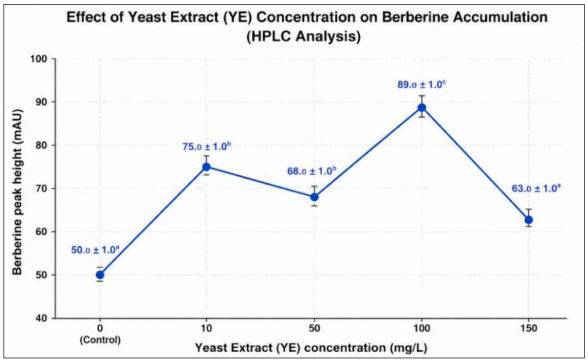


Figure 3.4. Effect Of Yeast Extract (YE) Concentration On Berberine Accumulation In *A. mexicana* Suspension Cultures As Determined By HPLC Peak Height (mAU)



The metabolic response curve exhibited a highly non-linear, "bell-shaped" (biphasic) trend. A significant surge in berberine accumulation was documented from the basal constitutive level of 50.0 ± 1.0 mAU in the untreated control to a maximum berberine accumulation of 89.0 ± 1.0 mAU at the 100 mg/L YE threshold. This represents a significant 1.78-fold (78%) increase in berberine accumulation over the control, indicating enhanced metabolic flux toward berberine biosynthesis. However, exceeding this optimal elicitor concentration led to a concentration-dependent decline in metabolic output, with peak heights falling to 63.0 ± 1.0 mAU at 150 mg/L YE, indicating suppressed metabolite accumulation.

This dose-dependent phenomenon is attributed to the role of biotic cell-wall elicitors present in the Yeast Extract as important signaling regulators. At the optimal moderate concentration (100 mg/L), complex carbohydrate fractions (beta-glucans, chitin oligomers) and glycoproteins derived from *Saccharomyces cerevisiae* function as fungal pathogen mimics. These elicitor molecules bind to transmembrane pattern recognition receptors (PRRs) on the cell surface of *A. mexicana*, triggering an intracellular defense cascade and an oxidative burst. This signaling leads to the upregulated transcription of rate-limiting biosynthetic enzymes, such as tyrosine decarboxylase and berberine bridge enzyme, in the benzylisoquinoline alkaloid (BIA) pathway. Conversely, supra-optimal concentrations (150 mg/L) induce cellular stress and feedback inhibition, which suppresses the cellular biosynthetic machinery and results in a reduced net berberine yield.

3.3. Biochemical Profiling of Antioxidant Capacity and Redox Homeostasis

The antioxidant capacity of Argemone mexicana L. cell suspension cultures was systematically evaluated following elicitation with varying concentrations of Yeast Extract (YE). The biofunctional potential of the methanolic extracts was quantified through the implementation of the DPPH radical scavenging assay. Basal antioxidant activity in the untreated control group (0 mg/L YE) was documented at 22.3 ± 0.4[a]%, representing the constitutive radical-quenching ability of the un-optimized culture. Upon the administration of moderate YE concentrations (10 and 50 mg/L), enhanced antioxidant activity relative to the control was observed. This induction of antioxidant potential reached maximum antioxidant activity at the 100 mg/L YE optimal elicitor concentration, where a maximum of 55.3 ± 0.6[c]% inhibition was recorded, representing a 2.48-fold increase over the control and the highest antioxidant performance within the evaluated YE elicitation series. However, as elicitor concentrations were increased to the supra-optimal level (150 mg/L), a statistically significant decline in scavenging

capacity occurred, with inhibition values receding to 30.6 ± 0.4[a]% (Table 3.3 and Figure 3.5). This reduction at the 150 mg/L threshold indicates stress-associated inhibition consistent with biotic cell-wall elicitor-mediated redox modulation.

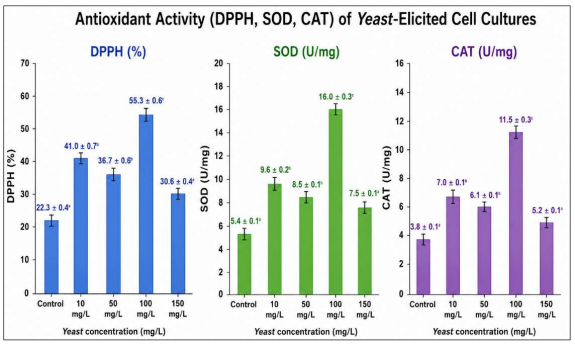
The total antioxidant capacity was further supported by the parallel, synchronized surges of the primary enzymatic defense components. Consistent with the radical scavenging trends, the activity of Superoxide Dismutase (SOD) increased from a basal 5.4 ± 0.1[a] U/mg to a maximum of 16.0 ± 0.3[c] U/mg at the 100 mg/L YE threshold, representing a near 3-fold induction. Similarly, Catalase (CAT) activity demonstrated a maximum response of 11.5 ± 0.3[c] U/mg at the 100 mg/L YE concentration compared to the untreated control value of 3.8 ± 0.1[a] U/mg. These results, summarized in Table 3.3, reveal that moderate concentrations of 10 mg/L and 50 mg/L successfully enhanced enzymatic activity (9.6 ± 0.2[b] and 8.5 ± 0.1[b] U/mg for SOD; 7.0 ± 0.1[b] and 6.1 ± 0.1[b] U/mg for CAT, respectively), while the sharp drop observed at 150 mg/L (7.5 ± 0.1[a] U/mg for SOD and 5.2 ± 0.1[a] U/mg for CAT) suggests a transition toward cellular toxicity or metabolic exhaustion. Different superscript letters (a, b, c) indicate significant differences determined by Tukey's HSD post-hoc test ($p \leq 0.05$).

Table 3.3. Effect Of Yeast Extract (YE) On Antioxidant Activity And Enzymatic Defense Responses In *Argemone mexicana* Callus Cultures

Treatment	DPPH (%)	SOD (U/mg)	CAT (U/mg)
Control (Untreated Cells)	22.3 ± 0.4[a]	5.4 ± 0.1[a]	3.8 ± 0.1[a]
10 mg/L YE	41.0 ± 0.7[b]	9.6 ± 0.2[b]	7.0 ± 0.1[b]
50 mg/L YE	36.7 ± 0.6[b]	8.5 ± 0.1[b]	6.1 ± 0.1[b]
100 mg/L YE	55.3 ± 0.6[c]	16.0 ± 0.3[c]	11.5 ± 0.3[c]
150 mg/L YE	30.6 ± 0.4[a]	7.5 ± 0.1[a]	5.2 ± 0.1[a]

Values represent Mean ± SD (n = 3). Different superscript letters denote statistically significant differences (Tukey, p ≤ 0.05).

Figure 3.5. Effect Of Yeast Extract Concentration On Antioxidant Defense Responses In *Argemone mexicana* Cell Cultures



A profound physiological correlation was identified between the secondary metabolic flux and the cellular defense response. The biphasic (bell-shaped) antioxidant scavenging curve was found to closely mirror the chromatographic berberine accumulation dynamics. Mechanistically, the perception of Yeast Extract's fungal pathogen mimics, such as beta-glucans and chitin oligomers, by host cell pattern recognition receptors (PRRs) triggers a transient, controlled intracellular oxidative burst and subsequent ROS accumulation. This accumulation acts as a critical secondary messenger that upregulates the transcription of endogenous antioxidant genes (SOD, CAT) and activates phenylpropanoid and benzylisoquinoline alkaloid (BIA) pathways. This coordinated redox regulation creates a protective biochemical shield that stabilizes cellular integrity, directly correlating with and facilitating the enhanced accumulation of the protoberberine alkaloid berberine. As a protoberberine alkaloid, berberine possesses a high redox potential and functions as an effective hydrogen-donating antioxidant, directly driving the enhanced DPPH scavenging performance in the elicited *A. mexicana* cultures. This synchronization suggests that the enhanced accumulation of berberine is an important component of the coordinated biochemical response required to maintain cellular homeostasis during the elicitor-induced oxidative burst.

3.4. Mechanistic Insights and Physiological Correlation

The synchronized biphasic response observed across both metabolic and antioxidant profiles in *Argemone mexicana* L. is fundamentally governed by the role of Yeast Extract (YE) as an important biotic fungal elicitor. At the optimal elicitor concentration (100 mg/L), the perception of Yeast Extract's fungal pathogen mimics (beta-glucans, chitin) and glycoproteins by host cell Pattern Recognition Receptors (PRRs) triggers an intracellular defense signaling cascade. This optimal elicitor concentration facilitates the activation of Mitogen-Activated Protein Kinase (MAPK) and calcium-linked signaling pathways, which are critical for the transcriptional

regulation of secondary metabolism. Specifically, the metabolic flux toward the protoberberine skeleton is driven by the elicitor-mediated upregulation of key rate-limiting enzymes in the benzylisoquinoline alkaloid (BIA) pathway, most notably tyrosine decarboxylase (TyDC) and berberine bridge enzyme (BBE). Under these optimized conditions, cellular vitality is maintained, and these cellular biosynthetic systems are successfully reprogrammed for enhanced berberine accumulation, which in turn enhances the radical scavenging potential of the cultures.

Conversely, the administration of the high 150 mg/L YE concentration triggers a physiological shift toward cellular stress. At this supra-optimal dosage, the intense elicitation instigates a state of acute oxidative stress that may contribute to membrane damage and activate feedback inhibition or metabolic exhaustion. This oxidative burden surpasses the collective buffering capacity of the enzymatic scavengers, SOD and CAT, as well as the non-enzymatic, berberine-mediated defense systems. The resulting decline in cell viability and the transition toward cellular senescence or death led to a statistically significant decrease in total alkaloid yield and a reduction in antioxidant performance.

This mechanistic bell-shaped response underscores the necessity of calibrated elicitor signaling for the biotechnological optimization of *A. mexicana*. The physiological correlation between enhanced berberine accumulation and peak radical scavenging capacity at the optimal YE concentration of 100 mg/L confirms that metabolic flux is specifically directed toward secondary metabolites that support cellular defense responses against induced oxidative bursts. Thus, the coordination between defense signaling and metabolic redirection provides a robust framework for the sustainable industrial production of berberine under controlled *in vitro* conditions, directly supporting the objectives of United Nations Sustainable Development Goal 12 (SDG 12) regarding responsible consumption and production.

4. Conclusion

The biotechnological optimization of berberine production within cell suspension cultures of *Argemone mexicana* L. represents a promising approach for sustainable and controlled berberine production, addressing issues associated with wild germplasm inconsistency and unsustainable harvesting. This research successfully established an efficient *in vitro* production system through the implementation of a 1.5 mg/L 2,4-D and 0.5 mg/L BAP hormonal combination, which facilitated a state of active cellular proliferation and achieved a maximum callus induction frequency of 95 ± 1.73% for highly friable, creamy-white callus lines. Based on standard growth behavior reported for plant cell suspension cultures, Day 20 was selected for elicitor application as a physiologically active stage often associated with enhanced responsiveness to biotic fungal cell-wall elicitors and secondary metabolite production, including benzylisoquinoline alkaloids.

The administration of an elicitor treatment utilizing 100 mg/L Yeast Extract (YE) was identified as the optimal concentration for enhanced berberine production, triggering a substantial 1.78-fold (78%) increase in berberine accumulation as validated by RP-HPLC analysis. This metabolic redirection was corroborated by a synchronized enhancement of enzymatic antioxidant defenses (SOD and CAT) and a maximum DPPH radical scavenging activity of 55.3 ± 0.6%, indicating enhanced antioxidant defense responses during increased berberine accumulation. This *in vitro* framework serves as a sustainable, climate-independent alternative to the ecologically taxing wild-harvesting of medicinal flora, contributing to biodiversity conservation while providing a sustainable source of berberine for future pharmaceutical applications. By providing a reliable source of berberine, this bioprocess directly aligns with the objectives of United Nations Sustainable Development Goal 12 (SDG 12) regarding responsible consumption and production.

5. References

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