European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Effect of Sodium Nitroprusside on Berberine Content in Argemone Mexicana Callus Cultures: An HPLC Analysis

Article History:

Received: 21-12-2025
Revised: 28-12-2025
Accepted: 28-12-2025
Published: 31-12-2025

Name of Author:
Pooja Vishwakarma[1], Sheetal Sharma[2] and Vijay D. Mendhulkar[3]

Affiliation: *[1]Research Scholar, Department of Biotechnology, The Institute of Science, Mumbai, Maharashtra, India*
[2]Research Scholar, Department of Botany, The Institute of Science, Mumbai, Maharashtra, India
[3]Professor, Department of Botany, The Institute of Science, Mumbai, Maharashtra, India

Corresponding Author:
Pooja Vishwakarma

Abstract: Argemone mexicana L. (Papaveraceae), commonly known as the Mexican poppy, is a medicinal plant valued for its isoquinoline alkaloid berberine, which exhibits antimicrobial, antioxidant, and hepatoprotective properties. However, its limited natural availability restricts large-scale extraction from wild sources. The present study aimed to evaluate the influence of sodium nitroprusside (SNP), a nitric oxide (NO) donor, as an abiotic elicitor on berberine biosynthesis under in vitro conditions. Callus cultures were established on Murashige and Skoog (MS) medium supplemented with optimized concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). Friable calli were transferred to liquid MS medium and treated with SNP (0, 2, 5, 10, and 15 mg/L). Berberine was extracted and quantified using high-performance liquid chromatography (HPLC) at 266 nm. SNP elicitation resulted in a concentration-dependent biphasic response, with berberine accumulation increasing up to an optimal SNP concentration, followed by a decline at higher levels. Maximum berberine accumulation was observed at 5 mg/L SNP, indicating effective stimulation at moderate NO levels. Enhanced berberine accumulation was accompanied by increased antioxidant activity, suggesting a positive association between metabolite enrichment and biofunctional potential. These findings indicate that NO-mediated elicitation can modulate secondary metabolism in A. mexicana cultures and highlight its potential as a sustainable biotechnological approach for improving berberine production under controlled conditions.

Keywords: Argemone mexicana, berberine, sodium nitroprusside, nitric oxide, elicitation, secondary metabolites, HPLC chromatography.

INTRODUCTION

Medicinal plants have long been central to traditional healing systems and continue to serve as indispensable resources in modern pharmaceutical research. Argemone mexicana L. (Papaveraceae), commonly known as the Mexican poppy, is an important medicinal plant with diverse therapeutic applications. It is widely distributed across tropical and subtropical regions. Traditionally, it has been used in Ayurveda, Siddha, and Unani systems to treat microbial infections, skin ailments, inflammation, jaundice, and liver-related disorders (Singh et al., 2020; Sharma et al., 2022).

The therapeutic significance of A. mexicana is largely attributed to its major isoquinoline alkaloid, berberine, a yellow benzylisoquinoline compound known for its antimicrobial, antioxidant, anti-inflammatory, hypoglycemic, and hepatoprotective properties (Imenshahidi & Hosseinzadeh, 2019; Jabeen et al., 2018). Berberine has also demonstrated promising potential in the management of metabolic disorders, cardiovascular diseases, and neurodegenerative conditions (Kumar et al., 2021). However, conventional extraction of berberine from field-grown plants is constrained by low metabolite

yields, environmental variability, and the risk of overharvesting, underscoring the need for sustainable and controlled in vitro production strategies.

In vitro culture techniques, including callus and suspension cultures, provide controlled, scalable, and reproducible platforms for secondary metabolite production (Ramakrishna & Ravishankar, 2011; Giri & Zaheer, 2016). Within these systems, elicitation—defined as the use of biotic or abiotic agents to simulate stress conditions—has emerged as an effective approach for enhancing metabolite biosynthesis (Zhao et al., 2005). Elicitors are known to activate stress-related signaling pathways, induce oxidative bursts, promote the generation of reactive oxygen species (ROS), and stimulate the transcriptional regulation of key biosynthetic enzymes involved in secondary metabolism (Hasanuzzaman et al., 2018).

Sodium nitroprusside (SNP), a commonly used nitric oxide (NO) donor, functions as an efficient abiotic elicitor capable of modulating physiological and metabolic pathways in plants (Mur et al.,

2013). Nitric oxide plays a crucial role as a signaling molecule in plants and is known to influence secondary metabolism through its involvement in upstream signaling cascades and enzyme regulation. Several studies have reported SNP-induced enhancement of secondary metabolites, including vinblastine in Catharanthus roseus (Patel et al., 2016), withanolides in Withania somnifera (Singh et al., 2017), and flavonoids in Capsicum annuum (Vardhini et al., 2003). In isoquinoline alkaloid biosynthetic pathways, NO has been reported to modulate the activity of enzymes such as tyrosine decarboxylase and berberine bridge enzyme, thereby influencing metabolic flux toward alkaloid accumulation (Neill et al., 2008; Benzekri et al., 2015). Moreover, NO-mediated stimulation of alkaloid biosynthesis has frequently been associated with enhanced antioxidant capacity, as many alkaloids, including berberine, possess inherent free radical- scavenging properties (Hasanuzzaman et al., 2021).

Despite the pharmacological importance of A. mexicana, limited information is available on the role of NO-mediated elicitation in regulating berberine biosynthesis in its in vitro cultures. Therefore, the present study aimed to evaluate the effects of different SNP concentrations on berberine accumulation in A. mexicana callus-derived suspension cultures. The specific objectives were as follows:

- To establish callus and suspension cultures of A. mexicana under controlled laboratory conditions.
- To evaluate the effect of SNP as an abiotic elicitor on berberine accumulation.
- Berberine content was quantified using high-performance liquid chromatography (HPLC).
- To assess the antioxidant activity of SNP-elicited cultures and examine its correlation with berberine accumulation.

This study provides insights into the NO-induced regulation of secondary metabolism and its associated enhancement of bio functional properties, such as antioxidant activity. It further demonstrates the potential of an in vitro culture-based approach as a sustainable platform for improving berberine production, with possible applications in the pharmaceutical and nutraceutical industries.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Healthy Argemone mexicana L. plants were collected from wild populations in the Konkan region of Maharashtra, India, during the post-monsoon season. The species was authenticated by comparison with a reference Specimen No (specimen no. SMA-1499) at the Blatter Herbarium in St. Xavier College, Mumbai. A voucher specimen was deposited in the Department of Botany Herbarium. Only mature, disease-free plants were used, and all experimental work was performed under aseptic conditions.

Surface Sterilization of Explants

Young shoot segments (2–3 cm) were washed under running tap water for 5–8 min, immersed in 1–2% Teepol for 3 min, and rinsed with sterile double-distilled water. The explants were then treated with 70% ethanol for 30–60 s in a laminar airflow cabinet and rinsed thrice with sterile water. Sterilized explants were trimmed to remove damaged portions before being inoculated.

Preparation of Culture Medium

Murashige and Skoog (MS) basal medium (Murashige & Skoog, 1962) supplemented with optimized concentrations of 2,4-D and BAP, based on preliminary trials, was used for the experiments. The medium contained 3% sucrose and 0.8% agar, with pH adjusted to 5.8 before autoclaving (121°C, 15 psi, 20 min). The media were aseptically poured into sterile glassware for culture initiation.

Callus Induction and Maintenance

Sterilized explants were inoculated on MS medium using sterile forceps and incubated at 25 ± 2°C under a 16/8 h light/dark cycle. Callus formation was observed within 10–12 days. Friable, cream- colored calli were subcultured every 3–4 weeks, whereas compact or necrotic calli were discarded to maintain uniform growth.

Establishment of Cell Suspension Cultures

Approximately 1 g of friable callus was transferred to

250 mL flasks containing 50 mL of liquid MS medium with optimized 2,4-D and BAP concentrations. Cultures were maintained at 25 ± 2°C on a rotary shaker (100 rpm) in the dark. Subculturing was performed biweekly to ensure viability and uniform cell dispersion.

Elicitation with Sodium Nitroprusside (SNP)
Ten-day-old suspension cultures were treated with SNP (0, 2, 5, 10, and 15 mg/L), a nitric oxide donor, and filter-sterilized through a 0.22 µm membrane. Treatments were performed in triplicate and incubated at 25 ± 2°C and 100 rpm in the dark for 48 h. After elicitation, the cultures were harvested for berberine estimation.

Harvesting and Extraction
The cultures were centrifuged at 5,000 rpm for 10 min, washed with sterile water, and freeze-dried. Approximately 100 mg of dried biomass was extracted with 10 mL acetonitrile–water (30:70 v/v) under sonication for 20 min. The extracts were centrifuged (10,000 rpm, 10 min), filtered through 0.22 µm membranes, and stored at 4°C in amber vials for analysis.

HPLC Quantification of Berberine
To ensure chromatographic consistency, both standard berberine solutions and SNP-treated samples were analyzed under identical mobile phase conditions consisting of acetonitrile and 0.2% acetic acid (42:58, v/v). The use of a uniform mobile phase minimized solvent-induced retention- time variations and enabled accurate comparisons between the calibration standards and the sample chromatograms. Chromatographic separation was achieved using a C18 reversed-phase column (250 mm × 4.6 mm, 5 µm). The mobile phase was delivered at a flow rate of 1.0 mL min^{-1} with an injection volume of 10 µL. The detection was performed at 266 nm. Under these conditions, the berberine standard exhibited a consistent retention time of 2.42–2.45 min.

Calibration and method validation were performed using standard berberine solutions at concentrations of 50–100 µg/mL. Calibration curves were constructed by plotting the peak intensity versus concentration, and linearity was evaluated using regression analysis. Method precision was assessed by calculating the percentage relative standard deviation (% RSD) of the retention time and peak intensity, and method sensitivity was determined by estimating the limit of detection (LOD) and limit of quantification (LOQ) from the standard deviation of the response and the slope of the calibration curve, in accordance with the ICH guidelines. The method demonstrated acceptable linearity, precision, and sensitivity, confirming its suitability for the quantitative estimation of berberine in elicited samples.

The regression equation described the calibration curve for berberine: peak intensity (mAU) = 1.16 × concentration (ppm) − 0.38.

DPPH Radical Scavenging Assay
Antioxidant activity was assessed using the DPPH radical-scavenging assay, as described by Brand-Williams et al. (1995). Cell culture extracts from various SNP treatments were mixed with DPPH, incubated in the dark at room temperature, and the absorbance was measured at 517 nm. Radical scavenging activity was expressed as the percentage inhibition relative to the control. Triplicate measurements were taken, and the results were correlated with the HPLC-measured berberine content to assess berberine's role in antioxidant activity.

ABTS Radical Scavenging Assay
ABTS radical-scavenging activity was evaluated using the $ABTS^{+}$• decolorization assay described by Re et al. (1999). The ABTS radical cation was generated by reacting ABTS with potassium persulfate, and the solution was incubated in the dark before use. Cell culture extracts from various SNP treatments were added to the $ABTS^{+}$• solution, and the decrease in absorbance was measured at 734 nm using a UV–visible spectrophotometer. Antioxidant activity was reported as the percentage of radical scavenging relative to the control. All assays were performed in triplicate, and the antioxidant response was correlated with the berberine levels determined by HPLC.

Contamination Management
All media, instruments, and glassware were sterilized before use. The laminar airflow chamber was disinfected with 70% ethanol and UV-irradiated before each session. Contaminated cultures were immediately discarded, and the experiments were repeated to ensure reproducibility.

Statistical Analysis
All experiments were performed in replicates, and the data are presented as mean ± standard deviation (SD). Statistical analysis included one-way analysis of variance (ANOVA) to assess significant differences among treatments. Tukey's multiple comparison test was used for post hoc analysis to identify differences among individual means. Results with p-values of less than 0.05 were deemed statistically significant.
Results

Callus Induction and Establishment of Suspension Cultures
The sterilization protocol employed in the present study was effective, resulting in more than 95% contamination-free explants. Young shoot explants of Argemone mexicana responded positively to the induction medium supplemented with an optimized combination of 2,4- dichlorophenoxyacetic acid (2,4-

D) and 6-benzylaminopurine (BAP).

Initial callus formation was observed within 10–12 days of inoculation, predominantly along the cut surfaces of the explants. The callus initially appeared compact and greenish-white, and with successive subcultures gradually developed into a friable, cream-colored mass. These friable calli were selected and used for the establishment of cell suspension cultures.

Upon transfer to liquid MS medium containing the same hormonal composition, the callus tissues readily disaggregated, leading to the formation of uniform cell suspensions. The cultured cells remained viable, exhibited active proliferation, and attained homogeneity after three successive subculture cycles. The established suspension culture system was subsequently utilized for elicitation experiments with sodium nitroprusside (SNP).

Optimization of Sodium Nitroprusside (SNP) Concentrations

Different concentrations of sodium nitroprusside (0, 2, 5, 10, and 15 mg/L) were tested to assess their effects on berberine biosynthesis in A. mexicana suspension cultures. The addition of SNP elicited visible physiological changes, including increased cell density and pigmentation intensity at lower to moderate concentrations. These changes are indicative of elicitor-induced modulation of secondary metabolite activity. At lower SNP concentrations (2 and 5 mg/L), cell viability remained high, accompanied by noticeable pigment intensification and enhanced metabolic activity. Maximum berberine accumulation was observed at 5 mg/L SNP, suggesting optimal elicitation at moderate nitric oxide levels. In contrast, higher SNP concentrations (10 and 15 mg/L) resulted in reduced biomass and diminished metabolite accumulation, likely due to stress-induced inhibitory effects at elevated NO levels.

HPLC Calibration and Standard Curve for Berberine

High-Performance Liquid Chromatography (HPLC) was used to quantify berberine in the control and SNP-treated cultures. The berberine standard exhibited a consistent retention time ranging from 2.421 to 2.468 min, indicating excellent chromatographic reproducibility. Calibration was performed using berberine standard solutions at concentrations of 50–100 ppm, yielding a linear relationship between concentration and peak intensity, with a high correlation coefficient ($R^2 = 0.998$). The regression equation obtained from the calibration curve was $y = 1.20x - 10.5$ (Tables 1 and 2).

Table 1. HPLC Retention Time and Peak Intensity of Berberine at Different Concentrations

Berberine 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 are expressed as mean ± SD (n = 3). Different superscript letters in the peak intensity column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

The method precision, expressed as the percentage relative standard deviation (%RSD), was ≤ 2.0% for peak intensity and retention time, demonstrating good analytical repeatability. Method sensitivity was assessed by calculating the limit of detection (LOD) and limit of quantification (LOQ), which were determined to be 1.8 ppm and 5.5 ppm, respectively, based on the standard deviation of the response and the slope of the calibration curve. The consistent retention times, excellent linearity, and satisfactory precision and sensitivity confirmed the suitability of the method for accurate quantitative estimation of berberine in elicited samples. The calibration data and validation parameters are summarized in Tables 1 and 2, respectively, and the standard berberine chromatogram is presented in Figure 1.

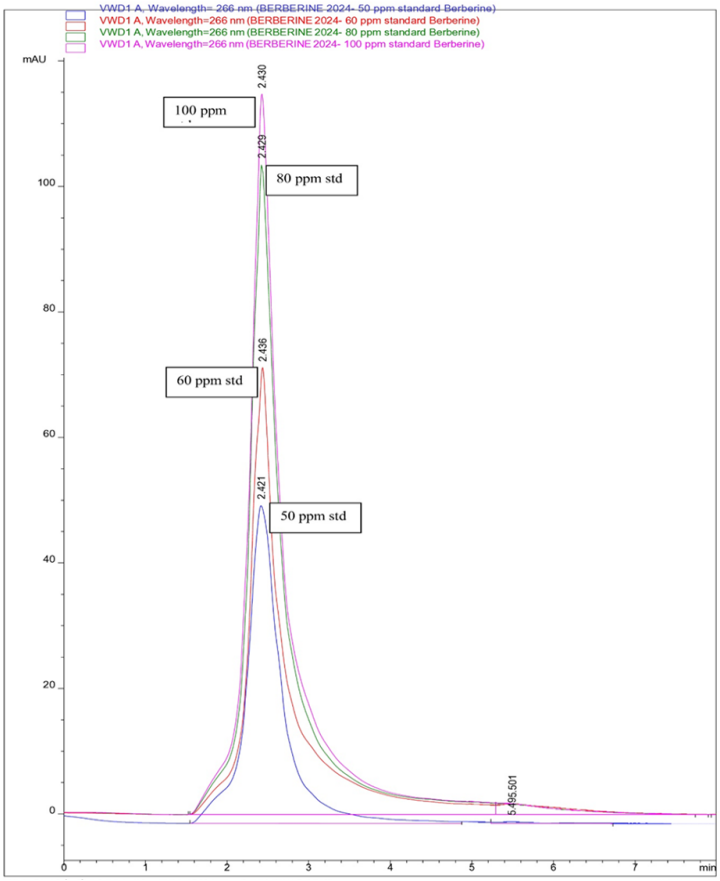
Table 2. Calibration and Validation Parameters for Berberine

Validation parameter	Result
Calibration curve	
Concentration range (ppm)	50–100

Regression equation	y = 1.20x − 10.5
Correlation coefficient (R^2)	0.998
Precision	
%RSD (n = 3)	≤ 2.0
Sensitivity	
Limit of detection, LOD (ppm)	1.8
Limit of quantification, LOQ (ppm)	5.5

The LOD and LOQ were calculated using the equations LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. The high retention time consistency and strong linear correlation validate the analytical method for accurate estimation of berberine in the elicited samples. The calibration curve for berberine is presented in Figure 1.

Figure 1. HPLC Chromatogram Overlay of Standard Berberine showing Retention Time and Detector Response (mAU)



HPLC Analysis of SNP-Treated Samples

The HPLC chromatograms of the control and SNP-treated samples exhibited distinct peaks at retention times corresponding closely to the berberine standard (2.42–2.46 min), confirming the presence of berberine in both control and elicited cultures. No major retention time drift was observed across treatments, indicating chromatographic stability under the applied conditions. Variations in peak intensity were recorded among different SNP concentrations, reflecting changes in berberine accumulation in response to elicitor treatment. The quantitative data derived from the HPLC analyses are presented in Table 3.

Table 3. Effect of Sodium Nitroprusside (SNP) Concentration on Berberine Accumulation

SNP concentration (mg/L)	Retention time (min)	Peak intensity (mAU)
0.0	2.42 ± 0.01[a]	50.0 ± 1.0[a]
2.0	2.46 ± 0.01[a]	85.0 ± 1.0[b]
5.0	2.43 ± 0.01[a]	92.0 ± 1.0[c]
10.0	2.45 ± 0.01[a]	79.0 ± 1.0[d]
15.0	2.43 ± 0.01[a]	66.0 ± 1.0[e]

Values are expressed as mean ± SD of three independent experiments (n = 3). Different superscript letters within the peak intensity column indicate significant differences according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

Berberine accumulation increased with SNP treatment up to an optimal concentration of 5 mg/L, beyond which a gradual decline in peak intensity was observed at higher SNP concentrations (10 and 15 mg/L). This pattern indicates a concentration-dependent biphasic response, wherein moderate nitric oxide levels stimulate secondary metabolite biosynthesis, while elevated levels exert an inhibitory effect, possibly due to elicitor-induced stress.

Comparative Analysis of SNP Treatments

The results demonstrated a concentration-dependent modulation of berberine biosynthesis following SNP elicitation. The control cultures exhibited a low peak intensity, reflecting basal- level berberine accumulation associated with normal metabolic activity. Treatment with a low SNP concentration (2 mg/L) resulted in a marked increase in berberine accumulation, while a further enhancement was observed at 5 mg/L SNP, indicating optimal elicitor-induced stimulation.

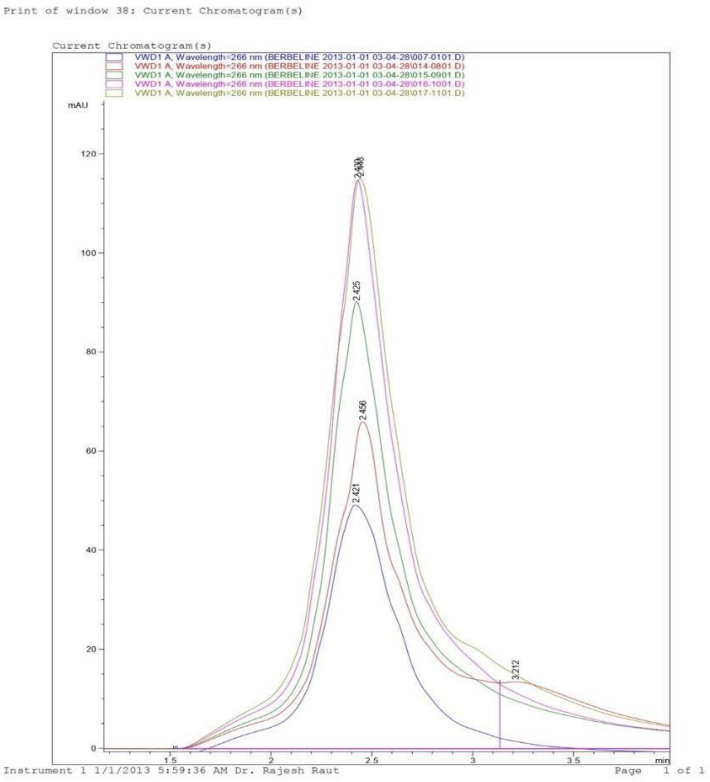
At higher SNP concentrations (10 and 15 mg/L), a decline in berberine accumulation was recorded, suggesting the onset of inhibitory effects at elevated nitric oxide levels. This biphasic response pattern indicates that moderate SNP concentrations effectively stimulate berberine biosynthesis, whereas excessive NO levels may impose metabolic stress, leading to reduced alkaloid production. Overall, the comparative analysis highlights the role of nitric oxide as a concentration-sensitive signaling molecule capable of modulating secondary metabolic pathways involved in alkaloid biosynthesis.

HPLC Chromatogram Description

Representative HPLC chromatograms of the berberine standard and SNP-treated samples (0, 2, 5, 10, and 15 mg/L) are presented in Figure 2. In the chromatograms, the berberine standard consistently exhibited a characteristic peak at approximately 2.43 min, which served as a reference for compound identification. The control sample showed a low-intensity peak near the same retention time, corresponding to basal-level berberine accumulation.

SNP-treated samples displayed clear variations in peak intensity across different concentrations. An increase in peak height was observed at lower to moderate SNP concentrations, with the most prominent and well-defined peak recorded at 5 mg/L SNP, indicating enhanced berberine accumulation under optimal elicitor conditions. At higher SNP concentrations (10 and 15 mg/L), a reduction in peak intensity was evident, suggesting a decline in berberine accumulation at elevated nitric oxide levels. Overall, the chromatographic profiles reflect a concentration- dependent biphasic response of berberine biosynthesis to SNP elicitation.

Figure 2. HPLC Chromatograms of Control and SNP-treated Argemone Mexicana Suspension Cultures showing Berberine Peaks recorded at 266 nm



Graphical Representation

The effect of SNP concentration on berberine accumulation is illustrated in Figure 3. A bar graph of SNP concentration (mg/L) versus berberine accumulation (expressed as HPLC peak intensity) demonstrated a clear concentration-dependent biphasic response. Berberine accumulation increased progressively from the control to lower and moderate SNP concentrations, reaching a maximum at 5 mg/L SNP, followed by a decline at higher concentrations (10 and 15 mg/L).

The results indicate that berberine production was optimally stimulated at moderate SNP levels, while elevated SNP concentrations resulted in reduced accumulation, suggesting stress-induced inhibition at higher nitric oxide levels. This trend highlights the concentration-sensitive role of SNP-mediated nitric oxide signaling in regulating berberine biosynthesis. No severe inhibitory effects were observed at lower SNP concentrations, indicating that the cultures tolerated moderate elicitor levels effectively. Overall, the graphical representation demonstrates that SNP acts as an effective elicitor within an optimal concentration range rather than inducing a linear dose- dependent response.

Figure 3. Effect of SNP Concentration on Berberine Accumulation in Argemone Mexicana Suspension Cultures as determined by HPLC Peak Height (mAU)

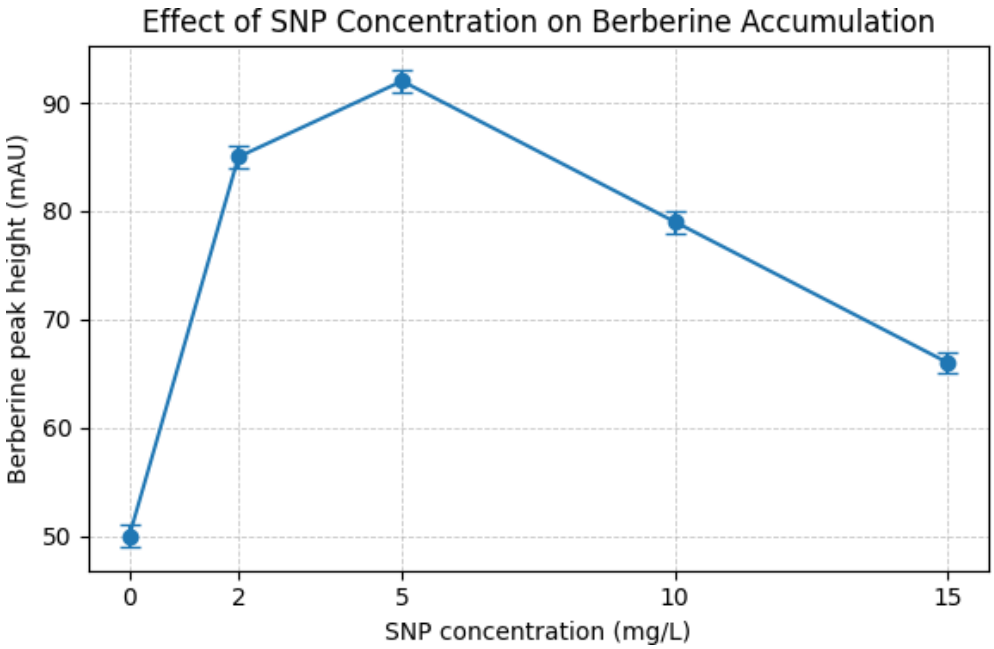
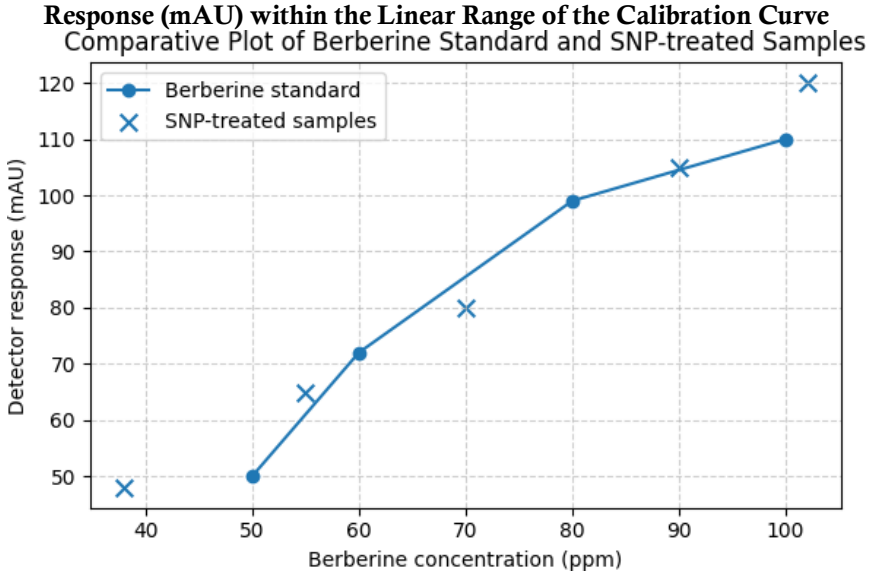


Figure 4. compares the berberine standard reference response with those of the SNP-treated samples. The graphical comparison illustrates enhanced berberine accumulation at moderate SNP concentrations, with the highest response observed at 5 mg/L SNP. At higher SNP concentrations, a gradual decline in berberine accumulation was evident, reinforcing the biphasic nature of the elicitor response.

Figure 4. Comparative Plot of Berberine Standard Calibration and SNP-Treated Samples showing Detector Response (mAU) within the Linear Range of the Calibration Curve

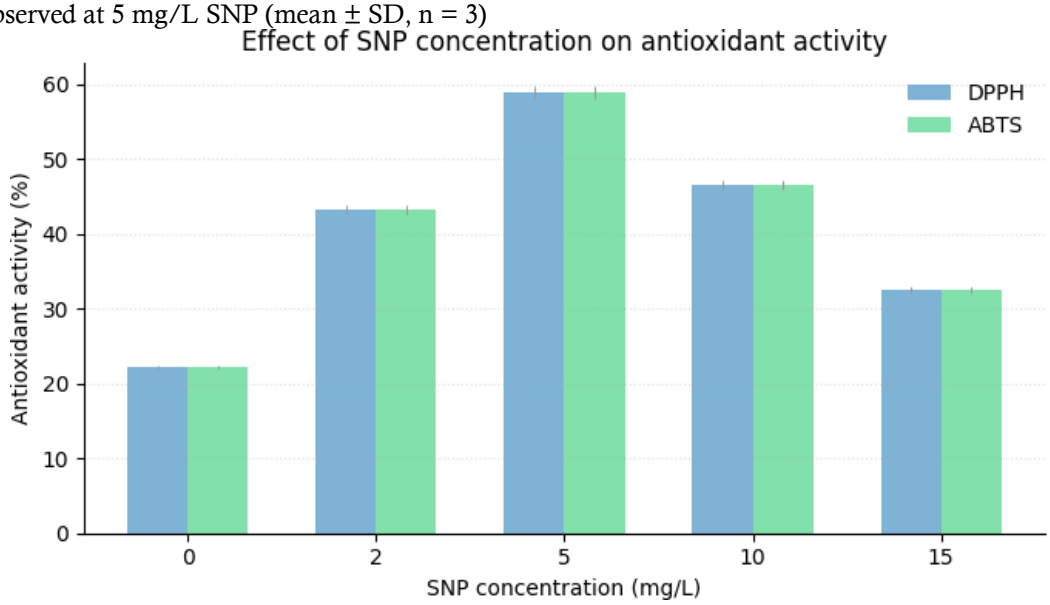


Effect of SNP Treatment on Antioxidant Activity (DPPH and ABTS Assays)

SNP-mediated elicitation resulted in a marked enhancement of antioxidant activity, as indicated by increased DPPH and ABTS radical-scavenging capacities in SNP-treated cultures compared to the control (Figure 5). Both assays exhibited a concentration-dependent biphasic response, with antioxidant activity increasing at lower to moderate SNP concentrations and reaching a maximum at 5 mg/L SNP. At higher SNP concentrations (10 and 15 mg/L), a decline in DPPH and ABTS radical-scavenging activity was observed, suggesting reduced antioxidant efficiency under elevated nitric oxide levels. The observed antioxidant trends closely paralleled the pattern of berberine accumulation, indicating a positive association between enhanced berberine levels and free radical-scavenging potential at optimal SNP concentrations. Overall, the results demonstrate that SNP-mediated nitric oxide signaling enhances antioxidant activity within a specific concentration range rather than inducing a linear dose-dependent response.

Figure 5. Effect of SNP Concentration on the Antioxidant Activity of Cell Culture Extracts as determined by DPPH

and ABTS Assays, showing a Concentration-Dependent Biphasic response with Maximum Radical-Scavenging Activity observed at 5 mg/L SNP (mean ± SD, n = 3)



DISCUSSION

The present study demonstrated that sodium nitroprusside (SNP), a nitric oxide (NO) donor, effectively modulated berberine production in Argemone mexicana L. callus-derived suspension cultures. SNP elicitation resulted in a concentration-dependent biphasic response, with berberine accumulation increasing at lower to moderate SNP concentrations and reaching an optimum at 5 mg/L SNP. At higher SNP concentrations, a decline in berberine accumulation was observed, indicating the onset of inhibitory effects at elevated nitric oxide levels.

Role of Nitric Oxide (NO) as a Signaling Molecule

Nitric oxide released from SNP is known to participate in ROS- and MAPK-linked signaling pathways, which are associated with the regulation of secondary metabolite biosynthesis in plants (Besson-Bard et al., 2008; Mur et al., 2013). In isoquinoline alkaloid biosynthetic pathways, nitric oxide has been reported to influence key enzymatic steps, including those involving tyrosine decarboxylase and berberine bridge enzyme, thereby modulating metabolic flux toward alkaloid accumulation.

Elicitor-Mediated Enhancement of Metabolite Production

Similar elicitor-mediated responses have been reported in Catharanthus roseus, Withania somnifera, and Capsicum annuum, where nitric oxide and other elicitors were shown to influence secondary metabolite accumulation under in vitro conditions (Jahin et al., 2016; Singh & Dwivedi, 2017; Vardhini & Rao, 2012). Based on these reports and the present findings, nitric oxide is likely to act as an upstream signaling molecule that modulates enzymatic activity and transcriptional regulation associated with berberine biosynthesis, rather than directly driving a linear enhancement of metabolite production.

Comparative and Mechanistic Insights

The observed results are consistent with previous studies reporting that nitric oxide can influence the accumulation of alkaloids, phenolics, and flavonoids in plant systems under elicitor-mediated conditions (Zhao et al., 2005; Benzekri et al., 2015). This consistency with earlier findings suggests that SNP-mediated nitric oxide signaling plays a regulatory role in modulating secondary metabolism in vitro, rather than acting as a sole determinant of metabolite enhancement.

Industrial and Biotechnological Relevance

The SNP-mediated modulation of berberine production highlights the potential of in vitro culture systems as a sustainable alternative to conventional field cultivation and wild harvesting. Such elicitor-based strategies can be further optimized in bioreactor systems by fine-tuning elicitor concentration and culture parameters to achieve reproducible metabolite yields (Ramakrishna & Ravishankar, 2011). In addition, integration of elicitation with metabolic engineering approaches and precursor feeding may enhance productivity and consistency, thereby supporting the use of A. mexicana as a controlled source of berberine for pharmaceutical and nutraceutical applications (Imenshahidi & Hosseinzadeh, 2019; Kumar et al., 2021). Overall, these elicitor-driven in vitro approaches align with current industrial interests in the regulated and sustainable production of high-value phytochemicals.

SNP-Induced Berberine Enhancement and Antioxidant Response

The enhancement of DPPH and ABTS radical-scavenging activities observed in SNP-treated cultures appears to be associated with nitric oxide (NO)-mediated modulation of secondary metabolism. Moderate SNP concentrations resulted in increased antioxidant activity, which coincided with enhanced berberine accumulation, whereas higher SNP levels showed a decline in both responses. Previous studies have demonstrated that exogenous application of NO donors, such as sodium nitroprusside, can activate redox-mediated defense pathways and influence secondary metabolite production in plant cell cultures, leading to altered levels of phenolics, flavonoids, and related bioactive compounds (Hasanuzzaman et al., 2021).

Berberine itself has been reported to exhibit notable antioxidant activity in DPPH and ABTS assays, supporting its contribution to free radical-scavenging potential. Taken together, the present findings suggest that SNP-induced NO signaling modulates berberine biosynthesis and antioxidant responses in a concentration-sensitive manner, with optimal enhancement occurring at moderate elicitor levels rather than through a linear dose-dependent effect.

Summary

The elicitor-mediated responses observed in this study highlight the role of nitric oxide (NO) as an important regulatory signal linking stress perception to changes in alkaloid biosynthesis. Previous studies have shown that NO donors, such as sodium nitroprusside, can modulate redox- mediated signaling networks and transcriptional regulation, thereby influencing the accumulation of secondary metabolites with associated biofunctional activities. In the present study, the parallel modulation of berberine accumulation and antioxidant capacity suggests that NO-driven signaling contributes to metabolic reprogramming in a concentration-sensitive manner rather than through a strictly linear enhancement.

Collectively, these findings indicate the potential of NO-mediated elicitation as a controllable in vitro strategy for influencing alkaloid production in A. mexicana. Future investigations should focus on kinetic profiling of NO effects, validation through standard-addition HPLC approaches, and scale-up studies using bioreactor-based suspension culture systems to further evaluate the applicability of this approach.

CONCLUSION AND FUTURE PROSPECTS

The present study demonstrated that sodium nitroprusside (SNP), as a nitric oxide (NO) donor, effectively modulates berberine production in Argemone mexicana suspension cultures under in vitro conditions. Berberine accumulation exhibited a concentration-dependent biphasic response, with enhanced accumulation at moderate SNP levels and a decline at higher concentrations, indicating the presence of an optimal elicitor range rather than a linear dose-dependent effect. This modulation of berberine biosynthesis was accompanied by corresponding changes in antioxidant activity, as reflected by DPPH and ABTS radical-scavenging assays, suggesting an association between alkaloid accumulation and biofunctional potential at optimal SNP concentrations.

The successful induction of friable callus and establishment of uniform suspension cultures further demonstrate the suitability of this in vitro system for studying isoquinoline alkaloid production. Overall, the findings indicate that NO-mediated elicitation represents a controllable strategy for influencing berberine biosynthesis and antioxidant responses in A. mexicana cultures, with potential applicability as an alternative to conventional plant harvesting when optimized appropriately.

Future prospects include detailed kinetic analyses of NO-mediated responses, validation of berberine quantification using standard-addition HPLC approaches, and scale-up studies employing bioreactor-based suspension culture systems. Such investigations would help to further evaluate the feasibility and reproducibility of NO-mediated elicitation for sustainable production of high-value phytochemicals.

Biotechnological and Industrial Implications

The ability to modulate berberine production through SNP-mediated elicitation highlights important possibilities for pharmaceutical, biotechnology, and natural products industries. Conventional extraction of berberine from wild plants often results in low and variable yields and may contribute to ecological imbalance due to habitat degradation and uncontrolled harvesting. In contrast, cell culture-based systems offer several advantages, including

- Year-round and climate-independent production.
- Controlled growth and improved reproducibility.
- Reduced environmental footprint.
- Potential for integration with bioreactors and semi-continuous production systems.

Moreover, SNP-mediated elicitation represents a promising and adaptable strategy for influencing berberine production under controlled in vitro conditions. The associated modulation of berberine-linked antioxidant activity may further enhance the functional value of cultured biomass for pharmaceutical and nutraceutical applications. With further optimization and scale-up validation, this approach could contribute to the sustainable utilization of A. mexicana as a renewable resource for

the production of berberine and related alkaloids.

Scientific Significance and Mechanistic Insights

These results support the role of nitric oxide as an important secondary messenger involved in coordinating plant stress responses and the regulation of secondary metabolism (Besson-Bard et al., 2008; Mur et al., 2013). In A. mexicana, nitric oxide released from SNP appears to influence alkaloid biosynthetic pathways in a concentration-sensitive manner rather than through a strictly dose-dependent response.

The observed enhancement of berberine accumulation at moderate SNP concentrations, followed by a decline at higher levels, suggests that NO-linked signaling can modulate metabolic flux toward secondary metabolite synthesis within an optimal elicitor range. This mechanistic insight highlights the regulatory, rather than absolute, role of nitric oxide in secondary metabolite biosynthesis under in vitro conditions.

Limitations and Future Directions

While the current study provides evidence for the involvement of SNP-mediated nitric oxide signaling in modulating berberine biosynthesis, further investigation is required to elucidate the underlying molecular mechanisms and to validate the observed responses more comprehensively. Future research should focus on the following aspects.

Molecular Characterization

- Transcriptomic and proteomic analyses should be conducted to identify specific genes, transcription factors, and regulatory proteins involved in NO-mediated modulation of secondary metabolism.
- Biochemical Pathway Analysis Detailed enzyme activity assays targeting key steps in isoquinoline alkaloid biosynthesis, such as tyrosine decarboxylase, berberine bridge enzyme, and associated methyltransferases, would help clarify pathway-level regulation.
- Optimization of Bioreactors Scaling up suspension cultures using airlift or stirred-tank bioreactor systems with optimized aeration, oxygen transfer, and agitation conditions could improve productivity, reproducibility, and process stability.
- Combined Elicitation Approaches: Integrating SNP with other elicitors, such as methyl jasmonate, salicylic acid, or yeast extract, may produce synergistic effects and further influence metabolite accumulation under controlled conditions.
- Metabolic Engineering: The application of metabolic engineering strategies, including precursor feeding and targeted manipulation of key biosynthetic genes, could be explored to enhance berberine accumulation and stability in cultured cells.

Environmental and Sustainable Impact

The development of in vitro biotechnological systems for metabolite production has the potential to contribute to environmental conservation by reducing reliance on wild plant harvesting. Argemone mexicana, often regarded as an invasive or underutilized species, may thus be explored as a renewable bioresource for controlled phytochemical production under laboratory conditions. Elicitor-based in vitro approaches can support emerging concepts of circular bioeconomy by promoting more efficient and regulated use of plant resources. In this context, such strategies are aligned with the objectives of the United Nations Sustainable Development Goals (SDG 12: Responsible Consumption and Production), emphasizing responsible and sustainable production of high-value phytochemicals.

REFERENCES

1. Ali, M. B., Hahn, E. J., & Paek, K. Y. (2006). Jasmonic acid and nitric oxide induce accumulation of phenolics in Panax ginseng roots. Plant Cell Reports, 25, 613–620.
2. Benzekri, H., Ferchichi, A., & Abbes, Z. (2015). Nitric oxide and plant secondary metabolism: Signal interactions and regulation mechanisms. Plant Science, 240, 1–10. https://doi.org/10.1016/j.plantsci.2015.08.012
3. Besson-Bard, A., Pugin, A., & Wendehenne, D. (2008). New insights into nitric oxide signaling in plants. Annual Review of Plant Biology, 59, 21–39. https://doi.org/10.1146/annurev.arplant.59.032607.092830
4. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT – Food Science and Technology, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5
5. Del Rio, L. A. (2015). ROS and RNS in plant signaling: Key regulators of stress responses.
6. Journal of Experimental Botany, 66, 2827–2839.
7. Ghosh, S., Saha, T., & Dey, P. (2019). Elicitor-induced secondary metabolite biosynthesis in plant cell and tissue culture: Recent trends and applications. Plant Biotechnology Reports, 13(4), 345–362. https://doi.org/10.1007/s11816-019-00560-3

8. Giri, C. C., & Zaheer, M. (2016). Chemical elicitors versus secondary metabolite production in vitro: A systematic review. Applied Microbiology and Biotechnology, 100(2), 571–582. https://doi.org/10.1007/s00253-015-7038-2
9. Hasanuzzaman, M., Nahar, K., & Fujita, M. (2018). Nitric oxide: A key player in plant abiotic stress tolerance. Plant Physiology and Biochemistry, 132, 459–472. https://doi.org/10.1016/j.plaphy.2018.09.001
10. Hasanuzzaman, M., Oku, H., Nahar, K., Bhuyan, M. H. M. B., Al Mahmud, J., Baluska, F., & Fujita, M. (2021). Nitric oxide-induced signaling and its role in plant stress tolerance and secondary metabolism. Plant Physiology and Biochemistry, 166, 264–279. https://doi.org/10.1016/j.plaphy.2021.06.009
11. Imenshahidi, M., & Hosseinzadeh, H. (2019). Berberine and its cardiovascular effects: A review. Iranian Journal of Basic Medical Sciences, 22(12), 1252–1266. https://doi.org/10.22038/ijbms.2019.34701.8295
12. Jabeen, A., Ahmad, S., & Khan, W. (2018). Antimicrobial potential of berberine and its derivatives: A review. Pharmacognosy Reviews, 12(24), 62–70. https://doi.org/10.4103/phrev.phrev_13_18
13. Jahin, M., et al. (2016). Elicitor-induced enhancement of secondary metabolites in
14. Catharanthus roseus cell cultures. Plant Physiology and Biochemistry, 104, 156–164.
15. Kumar, S., Sharma, S., & Singh, P. (2021). Therapeutic potential of berberine: A comprehensive review. Journal of Herbal Medicine, 26, 100404. https://doi.org/10.1016/j.hermed.2020.100404
16. Mur, L. A. J., Mandon, J., Persijn, S., Cristescu, S. M., Moshkov, I. E., Novikova, G. V., & Guo,
17. F. Q. (2013). Nitric oxide in plants: An assessment of the current state of knowledge. AoB Plants, 5, pls052. https://doi.org/10.1093/aobpla/pls052
18. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
19. Neill, S. J., Desikan, R., & Hancock, J. T. (2008). Nitric oxide signaling in plants. New Phytologist, 159(1), 11–35. https://doi.org/10.1046/j.1469-8137.2003.00724.x
20. Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., & Hancock, J. T. (2003). Hydrogen peroxide and nitric oxide as signalling molecules in plants. Journal of Experimental Botany, 54, 1237– 1247. https://doi.org/10.1093/jxb/erg027
21. Patel, S., Pandey, S., & Patel, N. (2016). Elicitation of vinblastine and ajmalicine using nitric oxide donors in Catharanthus roseus cell cultures. Plant Cell Reports, 35(4), 785–793. https://doi.org/10.1007/s00299-015-1904-8
22. Pati, R., Rath, S. K., & Mohapatra, P. K. (2019). Role of nitric oxide in plant metabolic pathways: A review. Plant Physiology Reports, 24(3), 324–337. https://doi.org/10.1007/s40502- 019-00466-2
23. Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior, 6(11), 1720–1731. https://doi.org/10.4161/psb.6.11.17613
24. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3
25. Sharma, D., Gupta, R., & Sharma, N. (2022). Traditional and modern perspectives of Argemone mexicana L.: Phytochemistry and pharmacology. Journal of Applied Research on Medicinal and Aromatic Plants, 31, 100389. https://doi.org/10.1016/j.jarmap.2022.100389
26. Sharma, M., Chandra, S., & Kumar, S. (2019). Elicitation: A biotechnological tool for enhanced secondary metabolite production. Plant Cell Reports, 38, 1–20.
27. Singh, D., & Dwivedi, T. (2017). Elicitor-mediated enhancement of secondary metabolites in medicinal plants. Plant Cell Biotechnology and Molecular Biology, 18, 20–30.
28. Singh, M., Mehta, S., & Rani, R. (2020). Phytochemical and pharmacological properties of Argemone mexicana: A review. Journal of Pharmacognosy and Phytochemistry, 9(3), 1895– 1903.
29. Singh, V. K., & Dwivedi, P. (2017). Role of nitric oxide in plant defense signaling: A review.
30. Plant Physiology Reports, 22(2), 140–152. https://doi.org/10.1007/s40502-017-0300-5
31. Vardhini, B. V., Rao, S. S. R., & Sujatha, E.

(2003). Effect of abiotic elicitors on secondary metabolite production in plant cell cultures. Plant Growth Regulation, 39(2), 111–115.

32. Wendehenne, D., Durner, J., & Klessig, D. F. (2004). Nitric oxide as a signal in plants. Current Opinion in Plant Biology, 7, 449–455. https://doi.org/10.1016/j.pbi.2004.05.002
33. Zhao, J., Davis, L. C., & Verpoorte, R. (2005). Elicitor signal transduction leading to secondary metabolite production. Biotechnology Advances, 23(4), 283–333. https://doi.org/10.1016/j.biotechadv.2005.01.003

