GMR The Original

COMPARATIVE ELICITOR-MEDIATED ENHANCEMENT OF PLUMBAGIN ACCUMULATION IN ADVENTITIOUS ROOT CULTURES OF PLUMBAGO INDICA AND PLUMBAGO AURICULATA

Vinay Kumar Hegde[1,2], Korse Keshava H[2], Penna Suprasanna[1], Abhishek Guldhe[1]*

[1] Amity Institute of Biotechnology, Amity University Maharashtra, Panvel, Mumbai -410206, Maharashtra, India.
[2] Bhandimane Life Science Research Foundation, Banavasi Road, Sirsi-581401, Uttara Kannada, Karnataka, India.
*Corresponding Author: Dr. Abhishek Guldhe, Email: abhiguldhe@gmail.com; asguldhe@mum.amity.edu

ABSTRACT

Plumbagin, a bioactive naphthoquinone produced by *Plumbago* species, has considerable pharmaceutical importance but its natural availability is limited by overharvesting and habitat degradation. The present study evaluated methyl jasmonate (MeJA) and chitosan as elicitors for enhancing plumbagin accumulation in non-transgenic adventitious root cultures of *Plumbago indica* and *Plumbago auriculata*. Adventitious roots were induced from leaf explants on half-strength Murashige and Skoog medium supplemented with indole-3-butyric acid and 1-naphthaleneacetic acid. Maximum root biomass was obtained on medium containing 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA. Four-week-old root cultures were treated for 7 days with MeJA (50–150 µM) and chitosan (100–200 mg L^{-1}), individually and in combination. Elicitation significantly increased plumbagin accumulation in both species compared with untreated controls. The combined treatment of 100 µM MeJA and 150 mg L^{-1} chitosan produced the highest plumbagin content, reaching 12.73 ± 0.20 µg mg^{-1} dry weight in *P. indica* and 10.14 ± 0.06 µg mg^{-1} dry weight in *P. auriculata*. These values corresponded to approximately 5.7-fold and 5.6-fold increases, respectively, over the controls. Across treatments, *P. indica* showed greater responsiveness than *P. auriculata*, indicating species-specific variation in elicitor-mediated metabolite accumulation. The enhanced response may be associated with the activation of jasmonate- and defense-related secondary metabolite pathways reported in *Plumbago* species. This study provides a simple, reproducible, and non-transgenic *in vitro* strategy for enhancing plumbagin accumulation and offers a useful framework for further molecular, metabolic, and process-level optimization.

KEYWORDS: Adventitious roots; Chitosan; Methyl jasmonate; Plumbagin; Secondary metabolites; Plant biotechnology; *Plumbago indica*; *Plumbago auriculata*

INTRODUCTION

Plumbago species are medicinally important plants known for the production of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), a naphthoquinone with reported antimicrobial, anti-inflammatory, anticancer, and antimalarial activities [1,2]. Among them, *Plumbago indica* L. and *Plumbago auriculata* Lam. are widely studied species with relevance in traditional medicine, phytochemistry, and plant biotechnology. *P. auriculata* is also commonly referred to by its synonym P. capensis in earlier literature [3]. Increasing demand for plumbagin, along with pressure on natural plant resources, has created a need for controlled and sustainable production systems.

In vitro culture techniques offer an important alternative for producing valuable plant secondary metabolites under controlled conditions. Root-based cultures are particularly useful because many bioactive compounds, including plumbagin, are naturally accumulated in underground tissues of *Plumbago* species. Hairy root, adventitious root, and regenerated shoot cultures have previously been explored for plumbagin production in different *Plumbago* species [4–9]. However, several earlier studies have focused on single species, transformed hairy root systems, or specific culture types, limiting direct comparison between species under uniform experimental conditions.

Elicitation is a widely used strategy to enhance secondary metabolite production in plant tissue cultures. Methyl jasmonate (MeJA) is a signalling molecule associated with jasmonate-mediated defence responses, while chitosan is a biotic elicitor that mimics pathogen-associated stress and activates plant defence-related metabolism [10]. Both elicitors are known to influence the biosynthesis of specialized metabolites by modulating stress-responsive and secondary metabolic pathways. In *Plumbago* and other medicinal plant systems, elicitor treatments have been reported to increase metabolite accumulation, including plumbagin production [6,8,11,12].

Although MeJA and chitosan have been studied individually in several plant culture systems, comparative evaluation of their individual and combined effects in non-transgenic adventitious root cultures of *P. indica* and *P. auriculata* remains limited. Such comparative studies are useful for identifying species-specific elicitor responses and for developing simple, reproducible, and cost-effective *in vitro* production systems. This is especially relevant for laboratories where advanced genetic transformation, omics analysis, or bioreactor facilities may not be readily available.

The present study was therefore designed to establish adventitious root cultures of *P. indica* and *P. auriculata* from leaf explants and to evaluate the effects of MeJA, chitosan, and their combined treatments on plumbagin accumulation. The study also compares the elicitation response of both species under identical culture conditions. By using a non-transgenic root culture system and readily available elicitors, this work provides an applied plant biotechnology approach for enhancing plumbagin accumulation and offers a foundation for future molecular, metabolic, and process-level optimization.

2. Materials and Methods

2.1 Plant material and establishment of adventitious root cultures

Healthy, mature plants of *Plumbago indica* L. and *Plumbago auriculata* Lam. were maintained under greenhouse conditions at Bhandimane Life Science Research Foundation (BLRF), Sirsi, Karnataka, India (PIN 581401; GPS: JR4W+P56). The plant materials were taxonomically authenticated, and voucher specimens were prepared and deposited in the institutional herbarium for future reference (Voucher No.: BLRF-PI-2020-01 and BLRF-PA-2020-01).

Young, fully expanded leaves were used as explants. The leaves were washed under running tap water for 10–15 min and treated with 2% (v/v) Tween-80 for 5 min, followed by rinsing with distilled water. Surface sterilization was carried out under aseptic conditions. Explants were treated sequentially with carbendazim (0.1% w/v) for 5–10 min, an antibiotic mixture of streptomycin and tetracycline for 5 min, 70% ethanol for 30–60 s, and either 2% sodium hypochlorite for 5–7 min or 0.1% mercuric chloride for 2–3 min, depending on contamination level. After each treatment, explants were rinsed 4–5 times with sterile distilled water.

Sterile leaf explants were inoculated on semi-solid half-strength Murashige and Skoog (MS) medium [13] supplemented with different combinations of indole-3-butyric acid (IBA; 0–2.0 mg L^{-1}) and 1-naphthaleneacetic acid (NAA; 0.2–1.0 mg L^{-1}). The medium contained 3% (w/v) sucrose and 0.8% (w/v) agar, and the pH was adjusted to 5.6 before autoclaving at 121°C for 15 min.

Cultures were maintained at 25 ± 2°C under a 16/8 h light/dark photoperiod with a light intensity of approximately 40–50 µmol m^{-2} s^{-1}. Each treatment consisted of five culture vessels (n = 5). After 4 weeks, the percentage of explants forming roots and root biomass expressed as fresh weight (FW) were recorded. The auxin combination producing maximum root biomass was selected for subsequent elicitation experiments.

2.2 Elicitor preparation and treatments

Four-week-old, well-developed adventitious root cultures of *P. indica* and *P. auriculata* grown on the optimized half-strength MS medium were used for elicitation studies. Uniform root clumps of approximately 1.5–2.0 g fresh weight were transferred to fresh medium containing methyl jasmonate (MeJA), chitosan, or their combinations.

MeJA was dissolved in 95% ethanol to prepare a stock solution and was filter-sterilized before being added aseptically to the medium. Chitosan was dissolved in 0.1% (v/v) acetic acid with continuous stirring, and the pH was adjusted to 5.6 before incorporation into the medium. Control treatments contained equivalent solvent concentrations to account for possible solvent effects.

MeJA was tested at 50, 100, and 150 µM, while chitosan was tested at 100, 150, and 200 mg L^{-1}. Combined treatments consisted of 50 µM MeJA + 100 mg L^{-1} chitosan, 100 µM MeJA + 150 mg L^{-1} chitosan, and 150 µM MeJA + 200 mg L^{-1} chitosan. These combinations were selected to represent low, intermediate, and high joint elicitor levels.

Root cultures were maintained under the same culture conditions described above. After 7 days of elicitation, roots were harvested, rinsed with distilled water, blotted dry, and processed for plumbagin estimation. The 7-day elicitation period was selected based on preliminary observations and earlier reports indicating enhanced plumbagin accumulation around this duration in *Plumbago* root cultures [6,8].

2.3 Plumbagin extraction and quantification

Harvested roots were shade dried at room temperature until constant weight and then ground into a fine powder. For each treatment, approximately 100 mg of dried root powder was extracted with methanol using a Soxhlet apparatus for 6 h at 60°C. The methanolic extracts were concentrated and re-dissolved in methanol for plumbagin estimation.

Plumbagin content was determined using a UV–visible spectrophotometric method with minor modifications from previously reported protocols [19,20]. Absorbance was recorded at 420 nm using methanol as blank. A standard calibration curve was prepared using authentic plumbagin at concentrations of 2, 4, 6, 8, and 10 µg mL^{-1}. The calibration curve showed a strong linear relationship within the tested range, with the regression equation $A_{420} = 0.0715C + 0.0050$ and coefficient of determination $R^2 = 0.998$, where A represents absorbance and C represents plumbagin concentration. Plumbagin content in root extracts was calculated from the standard curve and expressed as µg mg^{-1} dry weight (DW). Each treatment was analyzed using three independent biological replicates (n = 3).

2.4 Statistical analysis

All experiments were conducted using a completely randomized design. Data were expressed as mean ± standard error (SE). Root induction and biomass data were analyzed using analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test at $p < 0.05$.

For elicitation experiments, plumbagin content was analyzed to compare the effects of species and elicitor treatments. Significant differences among treatment means were determined using ANOVA followed by Tukey's HSD post hoc test at $p < 0.05$. Fold-change values were calculated relative to untreated control cultures to clearly express elicitor-mediated enhancement.

3. RESULTS

3.1 Establishment of adventitious root cultures

Leaf explants of *Plumbago indica* and *P. auriculata* responded differently to the tested combinations of IBA and NAA on half-strength MS medium. No adventitious root induction was observed on auxin-free medium, indicating that exogenous auxin supplementation was essential for rhizogenesis in both species.

In *P. indica*, the highest rooting response (100%) and maximum root biomass (5.28 ± 0.26 g FW) were recorded on medium supplemented with 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA. This response was statistically comparable to that obtained with 2.0 mg L^{-1} IBA and 0.2 mg L^{-1} NAA, but significantly higher than the responses observed under lower auxin combinations.

A similar pattern was observed in *P. auriculata*, where the combination of 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA produced 100% rooting and the highest biomass (5.49 ± 0.24 g FW). Lower auxin concentrations produced comparatively reduced biomass, indicating that an optimal IBA: NAA balance was required for efficient root development.

Based on these results, the combination of 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA was selected for subsequent elicitation experiments in both species. Representative stages of adventitious root induction and elicitor-treated root cultures are shown in Figure 1A and Figure 1B.

Table 1: Induction of roots from leaf explants of *P. indica* and *P. auriculata* after 4 weeks on half-strength MS medium supplemented with different combinations of IBA and NAA.

IBA (mg L^{-1})	NAA (mg L^{-1})	*P. indica* % response	*P. indica* root biomass (g FW)	*P. auriculata* % response	*P. auriculata* root biomass (g FW)
0.0	1.0	20	0.79 ± 0.06 d	20	0.68 ± 0.04 d
0.5	0.8	60	2.30 ± 0.13 c	40	2.17 ± 0.09 c
1.0	0.6	80	3.96 ± 0.32 b	80	3.52 ± 0.34 b
1.5	0.4	100	5.28 ± 0.26 a	100	5.49 ± 0.24 a
2.0	0.2	100	5.14 ± 0.17 a	100	5.12 ± 0.18 a

Values represent mean ± SE (n = 5). Data were subjected to two-way ANOVA followed by Tukey's HSD post hoc test. Different letters within a column indicate significant differences according to Tukey's HSD test at $p < 0.05$.

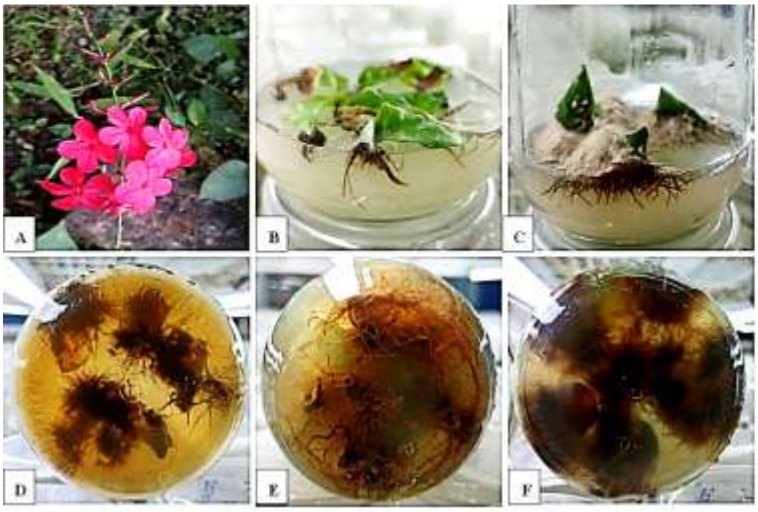

Figure 1A: Induction of adventitious roots from leaf explants and elicitor response in *in vitro* root cultures of *P. indica*.

(A) Characteristic red flowers of *P. indica* (B) Aseptically inoculated leaf explants after 7 days (C) Root biomass after 4 weeks of culture on optimized auxin medium

(D) Root cultures after 7 days of elicitation with MeJA (100 µM) (E) Root cultures after 7 days of elicitation with chitosan (150 mg L^{-1}) (F) Root cultures after 7 days of elicitation with MeJA (100 µM) + chitosan (150 mg L^{-1}).

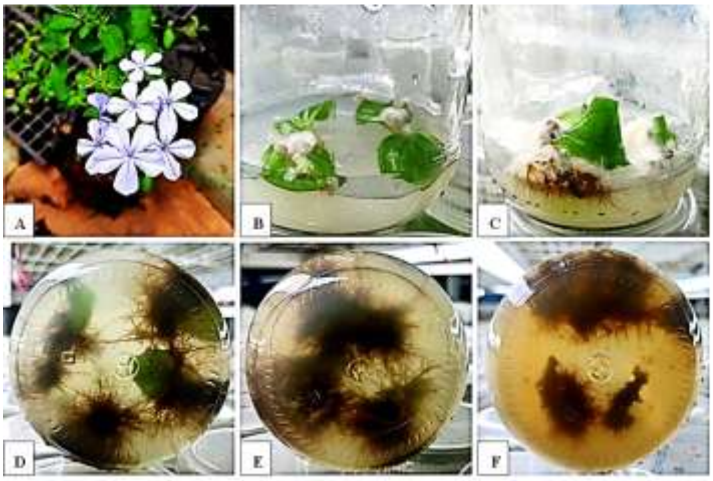

Figure 1B: Induction of adventitious roots from leaf explants and elicitor response in *in vitro* root cultures of *P. auriculata*.
(A) Characteristic blue flowers of *P. auriculata* (B) Aseptically inoculated leaf explants after 7 days (C) Root biomass after 4 weeks of culture on optimized auxin medium
(D) Root cultures after 7 days of elicitation with MeJA (100 µM) (E) Root cultures after 7 days of elicitation with chitosan (150 mg L^{-1}) (F) Root cultures after 7 days of elicitation with MeJA (100 µM) + chitosan (150 mg L^{-1}).

3.2 Effect of methyl jasmonate and chitosan on plumbagin accumulation

Elicitor treatments significantly enhanced plumbagin accumulation in adventitious root cultures of both *P. indica* and *P. auriculata* compared with untreated controls (Table 2).

In control cultures, plumbagin content was 2.24 ± 0.06 µg mg^{-1} DW in *P. indica* and 1.82 ± 0.02 µg mg^{-1} DW in *P. auriculata*. Treatment with methyl jasmonate alone resulted in a concentration-dependent increase in plumbagin accumulation up to 100 µM. At this concentration, plumbagin content reached 9.19 ± 0.64 µg mg^{-1} DW in *P. indica* and 7.10 ± 0.11 µg mg^{-1} DW in *P. auriculata*, representing approximately 4.1-fold and 3.9-fold increases, respectively, over the controls. Increasing MeJA concentration beyond 100 µM resulted in a reduction in plumbagin accumulation.

Chitosan treatment also significantly enhanced plumbagin accumulation. Among the tested concentrations, 150 mg L^{-1} chitosan was the most effective, producing 7.94 ± 0.10 µg mg^{-1} DW in *P. indica* and 6.83 ± 0.15 µg mg^{-1} DW in *P. auriculata*. These values corresponded to approximately 3.5-fold and 3.7-fold increases, respectively, compared with untreated controls. Lower and higher concentrations were comparatively less effective.

Combined application of methyl jasmonate and chitosan resulted in greater plumbagin accumulation than either elicitor applied individually. The combination of 100 µM MeJA and 150 mg L^{-1} chitosan produced the highest plumbagin levels observed in the study, reaching 12.73 ± 0.20 µg mg^{-1} DW in *P. indica* and 10.14 ± 0.06 µg mg^{-1} DW in *P. auriculata*. These values represented approximately 5.7-fold and 5.6-fold increases, respectively, relative to the controls. Other combined treatments also enhanced plumbagin accumulation, although to a lesser extent.

Across all elicitor treatments, *P. indica* consistently accumulated higher plumbagin levels than *P. auriculata*, indicating species-specific variation in elicitor responsiveness and secondary metabolite production potential under the tested culture conditions.

A bell-shaped response pattern was observed for both elicitors, with intermediate concentrations producing maximum plumbagin accumulation, whereas higher concentrations resulted in reduced metabolite levels. This pattern suggests that excessive elicitor stress may negatively affect metabolic activity and secondary metabolite biosynthesis.

The calibration curve used for plumbagin estimation exhibited excellent linearity ($R^2 = 0.998$), confirming the reliability of the spectrophotometric method within the tested concentration range (Figure 2).

Table 2: Plumbagin content (µg mg^{-1} DW) in root cultures of *P. indica* and *P. auriculata* after 7 days of elicitor treatment on half-strength MS medium.

MeJA (µM)	Chitosan (mg L^{-1})	*P. indica* plumbagin (µg mg^{-1} DW)	*P. auriculata* plumbagin (µg mg^{-1} DW)
0	0	2.24 ± 0.06 j	1.82 ± 0.02 j
50	0	5.38 ± 0.04 h	4.69 ± 0.03 h
100	0	9.19 ± 0.64 d	7.10 ± 0.11 d
150	0	8.10 ± 0.25 e	6.43 ± 0.11 f
0	100	4.56 ± 0.29 i	3.10 ± 0.11 i
0	150	7.94 ± 0.10 f	6.83 ± 0.15 e
0	200	6.21 ± 0.16 g	5.20 ± 0.12 g
50	100	10.24 ± 0.18 c	9.02 ± 0.07 c
100	150	12.73 ± 0.20 a	10.14 ± 0.06 a
150	200	11.57 ± 0.23 b	9.47 ± 0.18 b

Values represent mean ± SE (n = 3). Data were subjected to two-way ANOVA followed by Tukey's HSD post hoc test. Different letters within a column indicate significant differences according to Tukey's HSD test at $p < 0.05$.

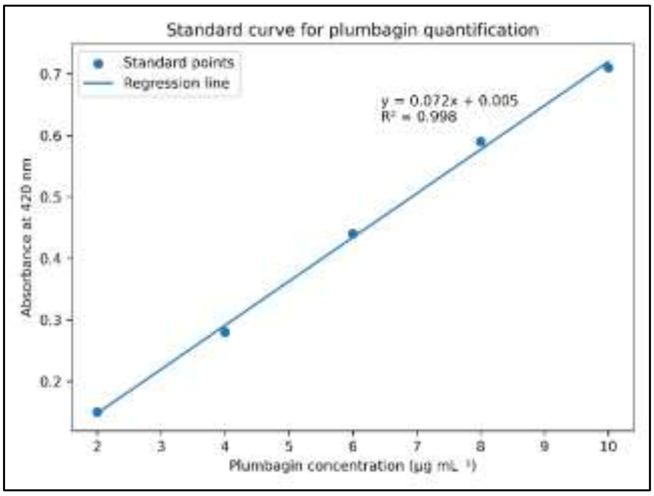

Figure 2. Standard calibration curve for plumbagin quantification using UV–visible spectrophotometry. Plumbagin standards (2–10 µg mL^{-1}) were measured at 420 nm, and a linear regression was obtained ($A_{420} = 0.0715C + 0.0050$; $R^2 = 0.998$), where A is absorbance and C is plumbagin concentration (µg mL^{-1}). The calibration curve was used to estimate plumbagin content (µg mg^{-1} dry weight) in root extracts of *P. indica* and *P. auriculata*.

4. DISCUSSION

The present study demonstrates that adventitious root cultures of *Plumbago indica* and *P. auriculata* provide effective *in vitro* platforms for elicitor-mediated enhancement of plumbagin accumulation. Adventitious root systems are particularly attractive for secondary metabolite production because they combine genetic stability, rapid growth, and the ability to accumulate root-associated bioactive compounds under controlled culture conditions. The successful establishment of root cultures in both species using 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA confirms the importance of auxin balance in promoting root biomass development and creating a suitable foundation for subsequent elicitation studies.

Both methyl jasmonate (MeJA) and chitosan significantly enhanced plumbagin accumulation when applied individually. MeJA is widely recognized as a signalling molecule involved in jasmonate-mediated defence responses and secondary metabolite biosynthesis. Activation of jasmonate signalling has been associated with increased expression of genes involved in specialized metabolic pathways in numerous medicinal plant species. Similarly, chitosan functions as a biotic elicitor that mimics pathogen-associated signals and stimulates defence-related metabolic responses. Previous studies have reported increased production of secondary metabolites following treatment with either MeJA or chitosan in root cultures, hairy roots, and regenerated tissues of medicinal plants, including members of the genus *Plumbago* [10,11,14].

In the present investigation, intermediate concentrations of both elicitors produced the highest plumbagin accumulation, whereas higher concentrations resulted in reduced metabolite levels. Similar bell-shaped responses have been reported in elicitation studies involving medicinal plants and are generally attributed to metabolic imbalance or excessive stress at elevated elicitor concentrations. Moderate elicitor levels appear to stimulate defence-associated metabolism efficiently, whereas excessive elicitation may redirect cellular resources toward stress adaptation rather than secondary metabolite biosynthesis.

A particularly important finding of this study was the enhanced response observed when MeJA and chitosan were applied together. The combined treatment of 100 µM MeJA and 150 mg L^{-1} chitosan produced the highest plumbagin accumulation in both species. Although formal interaction analysis was not performed, the greater response obtained under combined elicitation suggests that simultaneous activation of multiple defence-related signalling pathways may increase metabolic flux toward plumbagin biosynthesis. Similar observations have been reported in previous studies where combinations of elicitors produced greater metabolite accumulation than individual treatments alone.

The comparative evaluation of two *Plumbago* species under identical culture conditions revealed clear species-specific differences in elicitor responsiveness. Across all treatments, *P. indica* accumulated higher levels of plumbagin than *P. auriculata*. These differences may reflect variation in endogenous metabolic regulation, precursor availability, enzyme activity, or responsiveness to elicitor-mediated signalling pathways. Previous reports have also documented substantial variation in secondary metabolite accumulation among closely related medicinal plant species, emphasizing the importance of species selection when developing *in vitro* production systems.

The plumbagin levels obtained in the present study are comparable with those reported for several non-transgenic *in vitro* culture systems. While higher yields have been reported in certain optimized hairy root cultures, adventitious root cultures offer practical advantages, including simpler establishment procedures, reduced regulatory concerns, and broader applicability in laboratories lacking genetic transformation facilities. Consequently, the present system provides a useful alternative for laboratories seeking cost-effective approaches for secondary metabolite enhancement.

From a biotechnology perspective, the results demonstrate the potential of elicitor-based metabolic enhancement as a practical strategy for improving plumbagin accumulation in controlled culture systems. The use of non-transgenic adventitious roots, readily available elicitors, and simple analytical methods makes the protocol accessible and reproducible. Such systems may serve as valuable experimental platforms for future studies integrating molecular analysis, metabolomics, pathway characterization, bioreactor cultivation, and advanced process optimization.

Although plumbagin quantification was performed using a validated UV–visible spectrophotometric method, future studies incorporating chromatographic approaches such as HPLC or LC–MS would provide additional analytical specificity. Likewise, transcriptomic, enzymatic, and metabolomic investigations would contribute to a deeper understanding of the molecular mechanisms underlying elicitor-mediated enhancement of plumbagin biosynthesis.

Overall, the findings establish a comparative framework for evaluating elicitor responses in *Plumbago* species and demonstrate that dual elicitation with methyl jasmonate and chitosan represents an effective strategy for increasing plumbagin accumulation in adventitious root cultures. The study contributes to the development of sustainable plant biotechnology approaches for the production of valuable medicinal compounds and provides a foundation for future molecular and process-level improvements.

5. CONCLUSION

The present study successfully established adventitious root cultures of *Plumbago indica* and *Plumbago auriculata* and demonstrated that elicitation with methyl jasmonate and chitosan can significantly enhance plumbagin accumulation under controlled *in vitro* conditions. The optimized auxin combination of 1.5 mg L^{-1} IBA and 0.4 mg L^{-1} NAA supported efficient root induction and biomass development in both species, providing a suitable platform for elicitation studies.

Among the treatments evaluated, the combined application of 100 µM methyl jasmonate and 150 mg L^{-1} chitosan produced the highest plumbagin accumulation, resulting in approximately 5.7-fold and 5.6-fold increases in *P. indica* and *P. auriculata*, respectively, compared with untreated controls. The results further revealed species-specific differences in elicitor responsiveness, with *P. indica* consistently exhibiting higher plumbagin accumulation than *P. auriculata* under the tested conditions.

These findings highlight the potential of elicitor-mediated metabolic enhancement as an effective plant biotechnology approach for improving secondary metabolite production in non-transgenic root culture systems. The protocol developed in this study is simple, reproducible, and suitable for laboratories with limited infrastructure, thereby offering a practical framework for future optimization.

Future investigations integrating chromatographic validation, molecular characterization of biosynthetic pathways, metabolomic profiling, and bioreactor-based cultivation strategies may further improve the efficiency and commercial applicability of *in vitro* plumbagin production systems. Overall, the study provides a comparative and applied biotechnology perspective on plumbagin enhancement in *Plumbago* species and contributes to the development of sustainable *in vitro* production platforms for high-value medicinal compounds.

Acknowledgements

This manuscript forms part of the PhD thesis of the first author. The authors are grateful to Bhandimane Life Science Research Foundation (BLRF), Sirsi, Karnataka, India, for providing laboratory facilities and logistical support for plant tissue culture and phytochemical analysis.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Funding

The authors received no external funding for this research.

REFERENCES

1. Abdu A, Prakash A, Kondal R, Sharma S, Bhagat M, Pal R, Singh H, Singh B, Kaur S. Comprehensive review on *Plumbago indica*: traditional, pharmacological insights and conservation strategies. Journal of Applied Pharmaceutical Science. 2024; 14:1–16.
2. Priyanjani HASA, De Silva GRC, Sangwan SV. Propagation, phytochemistry and pharmacology of *Plumbago indica*: a review. Journal of Pharmaceutical Research International. 2021; 33:188–202.
3. Plants of the World Online. *Plumbago auriculata* Lam. Royal Botanic Gardens, Kew; 2025. Available from: http://www.plantsoftheworldonline.org
4. Gangopadhyay M, Sircar D, Mitra A, Bhattacharya S. Hairy root culture of *Plumbago indica* as a potential source for plumbagin. Biologia Plantarum. 2008; 52:533–537.
5. Martin KP, Sabovljevic A, Madassery J. High-frequency transgenic plant regeneration and plumbagin production through methyl jasmonate elicitation from hairy roots of *Plumbago indica* L. Journal of Crop Science and Biotechnology. 2011; 14:205–212.
6. Gangopadhyay M, Dewanjee S, Bhattacharya S. Enhanced plumbagin production in elicited *Plumbago indica* hairy root cultures. Journal of Bioscience and Bioengineering. 2011; 111:706–710.
7. Jose B, Silja PK, Pillai DB, Satheeshkumar K. *In vitro* cultivation of hairy roots of *Plumbago* rosea L. in a customized reaction kettle for the production of plumbagin—an anticancer compound. Industrial Crops and Products. 2016; 87:89–95.
8. Silja PK, Satheeshkumar K. Establishment of adventitious root cultures from leaf explants of *Plumbago* rosea and enhanced plumbagin production through elicitation. Industrial Crops and Products. 2015; 76:479–486.

9. Beigmohamadi M, Movafeghi A, Jafari S, Sharafi A. Efficient *in vitro* organogenesis, micropropagation, and plumbagin production in *Plumbago* europaea L. *In vitro* Cellular & Developmental Biology - Plant. 2021; 57:820–830.
10. Alcalde MA, Pérez-Matas E, Palazón J, Bonfill M. Biotic elicitors in adventitious and hairy root cultures: a review from 2010 to 2022. Molecules. 2022; 27:5253.
11. Li Y, Zhang S, Wang M, Guo L, Liu Y. MeJA-induced hairy root cultures in *Plumbago auriculata* Lam. for enhanced plumbagin production. Frontiers in Plant Science. 2024; 15:1411963.
12. Jirakiattikul Y, Ruangnoo S, Sangmukdee K, Chamchusri K, Rithichai P. Enhancement of plumbagin production through elicitation in *in vitro*-regenerated shoots of *Plumbago indica* L. Plants. 2024; 13:1450.
13. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962; 15:473–497.
14. Beigmohamadi M, Movafeghi A, Jafari S, Sharafi A. The influence of elicitors on plumbagin production and polyketide synthase gene expression in cultures of *Plumbago* europaea L. *In vitro* Cellular & Developmental Biology - Plant. 2025;61(6):1186–1201.
15. Jaisi A, Panichayupakaranant P. Enhanced plumbagin production in *Plumbago indica* root culture by simultaneous and sequential dual elicitations using chitosan with L-alanine and methyl-β-cyclodextrin. Bioresources and Bioprocessing. 2020; 7:10.
16. Chayjarung P, Poonsap W, Pankaew C, Inmano O, Kongbangkerd A, Limmongkon A. Using a combination of chitosan, methyl jasmonate, and cyclodextrin as an effective elicitation strategy for prenylated stilbene compound production in Arachis hypogaea L. hairy root culture. Plant Cell, Tissue and Organ Culture. 2021; 147:117–129.
17. Santra I, Ghosh B. Sustainable production of plumbagin via meta-topolin-mediated high frequency regeneration of genetically true-to-type *Plumbago* zeylanica L. 3 Biotech. 2023; 13:225.
18. Katoch K, Gupta S, Gupta AP, Goyal P, Devi R, Dey A, Pandey DK. Biotic elicitation for enhanced production of plumbagin in regenerated shoot cultures of *Plumbago* zeylanica using response surface methodology. Plant Cell, Tissue and Organ Culture. 2022; 151:605–615.
19. Israni SA, Kapadia NS, Lahiri SK, Yadav GK, Shah MB. A UV–visible spectrophotometric method for the estimation of plumbagin. International Journal of ChemTech Research. 2010; 2:856–859.
20. Adusei EBA, Adosraku RK, Oppong-Kyeyeky J, Amengor CDK. Investigation of acid–base indicator property of plumbagin from *Plumbago* zeylanica Linn. International Journal of Analytical Chemistry. 2019; 2019:4061927.
21. Arya SS, Lenka SK, Cahill DM, Rookes JE. Designer nanoparticles for plant cell culture systems: moving nanoelicitation from exploratory investigations to a commercially viable strategy. BioEssays. 2021;43: e2100081.