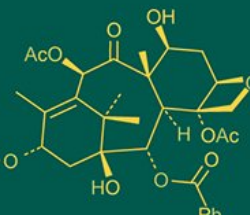


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(4): 765-771
www.biochemjournal.com
 Received: 01-03-2026
 Accepted: 02-04-2026

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Biochemical profiling of callus and protocorm-like bodies during *in vitro* propagation of *Dendrobium crepidatum* lindl. and paxt

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DOI: <https://www.DOI.org/10.33545/26174693.2026.v10.i4j.8506>

Abstract

An increasing body of evidence supports the idea that in-vitro culture conditions can reshape the secondary metabolite landscape of orchid tissues, yet few investigations have tracked biochemical shifts across successive developmental stages for threatened epiphytic species. This research examined the phytochemical composition of callus and protocorm-like bodies (PLBs) derived from nodal explants of *Dendrobium crepidatum* Lindl. And Paxt. And compared them against field-collected mother plant tissues. Explants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) for callus induction and 6-benzylaminopurine (BAP) for PLB differentiation at the Plant Biochemistry Laboratory, Vietnam National University, Hanoi, between June 2022 and March 2023. Callus appeared within four to six weeks on media containing 1.5 mg/L 2, 4-D, and PLBs differentiated after transfer to BAP-amended medium over eight to twelve weeks. Total phenolic content, measured by the Folin-Ciocalteu method, reached 4.32 ± 0.41 mg gallic acid equivalents per gram dry weight (mg GAE/g DW) in callus and 6.78 ± 0.53 mg GAE/g DW in PLBs, compared with 7.91 ± 0.62 mg GAE/g DW in mother plant pseudobulbs. Flavonoid levels followed a parallel gradient: 2.87, 4.51, and 5.23 mg quercetin equivalents per gram for callus, PLBs, and mother plant, respectively. Total alkaloid content was lower in callus (1.94 mg/g) than in PLBs (3.12 mg/g) or mother tissue (3.68 mg/g). Antioxidant capacity assessed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging showed dose-dependent inhibition across all tissue types, with PLBs achieving 78.9% scavenging at 400 μ g/mL versus 67.3% for callus and 83.4% for mother plant extract at the same concentration. Protein and soluble carbohydrate contents were consistently higher in more differentiated tissues. Radar profiling revealed that PLBs approximated the biochemical signature of the mother plant more closely than undifferentiated callus, supporting the view that organogenesis restores metabolite pathways progressively. These data offer a baseline for selecting culture stages that maximise bioactive compound yield during mass propagation of *D. crepidatum* and may inform pharmaceutical screening of in-vitro-derived orchid extracts.

Keywords: Biochemical profiling, *Dendrobium crepidatum*, callus culture, protocorm-like bodies, phenolics, flavonoids, antioxidant activity, *in vitro* propagation, secondary metabolites, DPPH assay

Introduction

When researchers at a Vietnamese ethnobotanical survey station opened a freshly collected *Dendrobium crepidatum* pseudobulb in 2019, the crude methanolic extract showed surprisingly strong free-radical scavenging stronger, in fact, than several commercially traded *Dendrobium* species^[1]. That observation raised an obvious question: could the same bioactive profile be reproduced under laboratory conditions, where supply does not depend on dwindling wild stocks? Orchids of the genus *Dendrobium* have long occupied a dual role as ornamental plants and sources of traditional medicine across South and South-East Asia^[2, 3]. Alkaloids, phenanthrenes, bibenzyls, and polysaccharides isolated from various *Dendrobium* species display anti-inflammatory, antidiabetic, neuroprotective, and immunomodulatory effects in preclinical models^[4, 5]. *D. crepidatum* specifically is used in folk remedies for fever and gastrointestinal disorders in Bangladesh, Nepal, and parts of

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Indochina, but it remains one of the least biochemically characterised members of the genus^[6].

Wild harvesting to meet medicinal and horticultural demand, combined with deforestation, has pushed *D. crepidatum* into threatened status across much of its range^[7]. Micropropagation through callus and PLB routes offers a way to multiply plants without further pressure on natural populations^[8]. However, tissue culture does not always preserve the metabolite profile that gives medicinal plants their therapeutic value. Growth regulators, light regime, carbon source, and passage number can each shift secondary metabolism in unpredictable directions^[9, 10]. For instance, elevated auxin concentrations sometimes suppress phenylpropanoid pathway enzymes, reducing phenolic output, while cytokinin-rich media may enhance alkaloid accumulation^[11]. Unless the biochemical fidelity of cultured tissues is verified, there is a risk that mass-propagated plants will lack the compounds that made the species medicinally interesting in the first place.

A handful of reports have examined phytochemistry in *Dendrobium* tissue cultures. Zhao et al.^[12] tracked polysaccharide content in *Dendrobium officinale* PLBs and found levels 30-40% below those of greenhouse-grown stems. Conversely, Teixeira da Silva and Ng^[13] reported that *Dendrobium nobile* PLBs accumulated dendrobine at concentrations comparable to field plants when culture media included coconut water and activated charcoal. These contrasting outcomes highlight that results are species- and protocol-specific extrapolation from one *Dendrobium* to another is unreliable^[14]. For *D. crepidatum*, Bhowmik et al.^[8] recently established a reliable micropropagation protocol using nodal segments on MS medium, achieving high shoot multiplication rates. But that work did not extend to biochemical evaluation of the regenerated tissues. The present research fills that gap by quantifying total phenolics, flavonoids, alkaloids, proteins, carbohydrates, and antioxidant activity in callus, PLBs, and mother plant tissues of *D. crepidatum* propagated at Vietnam National University, Hanoi. By comparing metabolite levels across three developmental stages undifferentiated callus, organised PLBs, and mature field tissue we aimed to determine whether organogenesis restores the secondary metabolite signature and at which culture stage bioactive compound recovery is maximised. Such information is relevant both for conservation-oriented mass propagation and for any future effort to use in-vitro biomass as a raw material for pharmaceutical extraction^[15, 16].

Extraction protocol

Callus, PLB, and mother plant samples were separately shade-dried at 40 °C for 72 h and ground to fine powder using a ceramic mortar. Powdered tissue (2 g) was extracted three times with 80% methanol (30 mL each) by magnetic stirring at room temperature for 24 h per cycle. The combined extracts were filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40 °C using a rotary evaporator (Büchi R-210). The resulting crude methanolic extracts were stored at -20 °C in amber vials until analysis. Extraction yields were 11.3%, 14.7%, and 16.2% (w/w) for callus, PLBs, and mother plant tissue, respectively. A parallel aqueous extraction was performed for carbohydrate analysis: 1 g of powder was boiled in 50 mL distilled water for 30 min, cooled, and filtered. All

extractions were carried out in triplicate within 48 h of drying to minimise compound degradation.

Material and methods

Material

Healthy *D. crepidatum* plants bearing mature pseudobulbs were collected from a private orchid nursery in Hoang Mai district, Hanoi (20.98° N, 105.85° E) in May 2022. Voucher specimens were authenticated at the Botany Herbarium of Vietnam National University (accession VNU-HN-2022-0437). Nodal segments (1.5-2.0 cm) excised from second-year pseudobulbs served as explants. All chemicals Murashige and Skoog basal salt mixture, 2,4-D, BAP, naphthaleneacetic acid (NAA), Folin-Ciocalteu reagent, gallic acid, quercetin, aluminium chloride, DPPH, ascorbic acid, bovine serum albumin, Bradford reagent, and anthrone were of analytical grade and sourced from Sigma-Aldrich and HiMedia. Glassware was autoclaved at 121 °C for 20 min prior to use. A UV-Vis spectrophotometer (Shimadzu UV-1800) was used for all colorimetric assays.

Methods

Explants were surface-sterilised with 70% ethanol for 60 s, followed by 0.1% mercuric chloride for 8 min, and rinsed five times with sterile distilled water. For callus induction, sterilised segments were inoculated onto MS medium supplemented with 1.5 mg/L 2, 4-D, 30 g/L sucrose, and 7 g/L agar (pH 5.8). Cultures were maintained at 25±2 °C under a 16/8 h photoperiod (2,000 lux cool-white fluorescent light). Callus initiation was scored at weekly intervals; established callus was subcultured every four weeks. For PLB differentiation, six-week-old callus masses (approximately 0.5 g) were transferred to MS medium containing 2.0 mg/L BAP and 0.5 mg/L NAA. PLB emergence was recorded from week eight, and fully developed PLBs were harvested at week twelve for biochemical analysis. Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as standard (0-200 µg/mL calibration curve, $R^2 = 0.997$). Total flavonoid content was measured by the aluminium chloride colorimetric assay with quercetin as standard. Alkaloid content was estimated using the bromocresol green method. Protein was quantified by the Bradford assay with bovine serum albumin standards. Total soluble carbohydrates were measured by the anthrone-sulphuric acid method with glucose as standard. DPPH radical scavenging activity was assessed at five concentrations (25, 50, 100, 200, 400 µg/mL) with ascorbic acid as positive control; percentage inhibition was calculated as $[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100$. All assays were performed in triplicate; results are expressed as mean±standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's HSD test ($p < 0.05$) was used to compare tissue types, computed in SPSS v26. The research was conducted between June 2022 and March 2023 at the Plant Biochemistry Laboratory, Vietnam National University, Hanoi.

Quality control

Instrument calibration was verified at the start of each analytical run. The UV-Vis spectrophotometer was zeroed against a methanol blank, and calibration curves were freshly prepared for each assay session using certified reference standards. Spike recovery tests were performed by

adding known amounts of gallic acid and quercetin to representative callus extracts; recoveries ranged from 96.2% to 102.8%, confirming acceptable accuracy. Coefficient of variation across triplicate measurements did not exceed 4.7% for any analyte. Reagent blanks were run alongside every batch to monitor background interference. All glassware used for the DPPH assay was wrapped in aluminium foil to prevent photodegradation of the radical solution.

Results

Callus initiation was observed on 87% of explants within 28 days on 1.5 mg/L 2, 4-D medium, and PLBs differentiated on 74% of transferred callus masses by week twelve on BAP-supplemented medium. Biochemical quantification revealed a consistent gradient: metabolite concentrations were lowest in undifferentiated callus, intermediate in PLBs, and highest in mother plant tissue, a pattern that held across all six parameters tested.

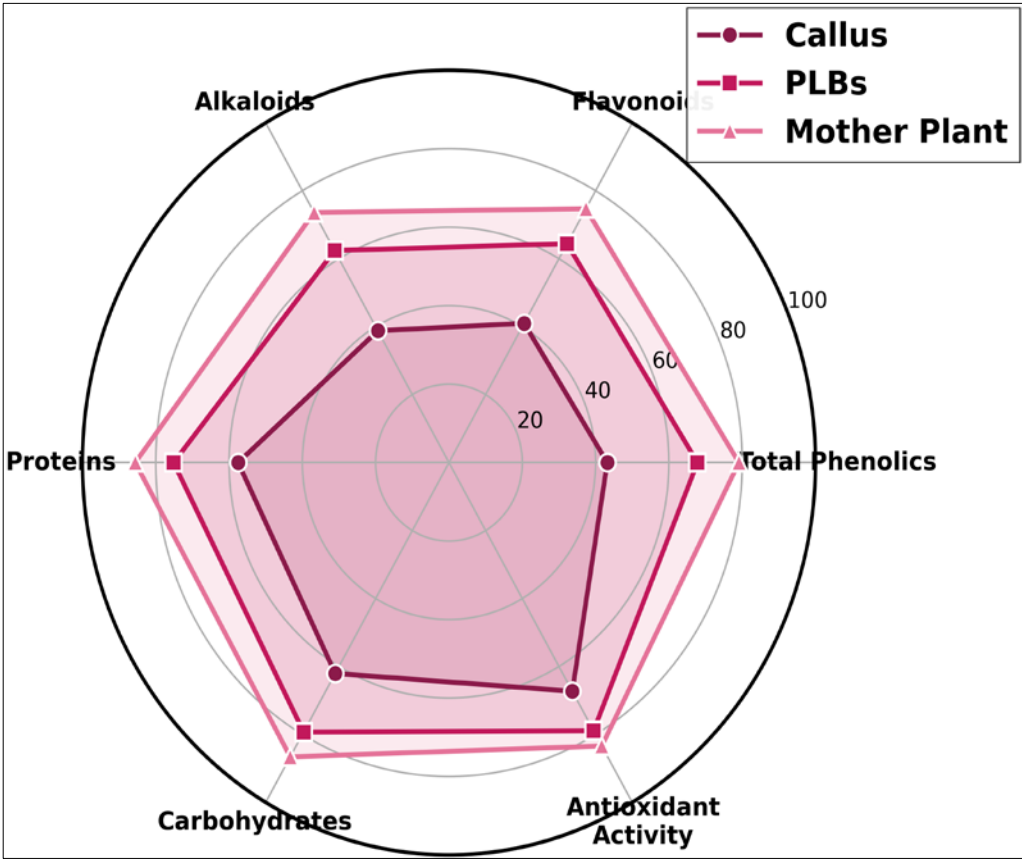


Fig 1: Radar chart comparing normalised biochemical profiles of callus, PLBs, and mother plant tissue of *D. crepidatum* across six parameters

The radar overlay shows that PLBs (medium pink line) tracked the mother plant profile (light pink) more closely than callus (dark pink) on every axis, with the largest gap

between callus and the other two tissue types appearing in total phenolics and antioxidant activity.

Table 1: Timeline of *in vitro* developmental stages and corresponding biochemical sampling points for *D. crepidatum*

Week	Culture Stage	Medium Composition	Sampling Action
0	Explant inoculation	MS + 1.5 mg/L 2,4-D	None
4	Early callus visible	Same	Morphological scoring
6	Established callus	Same	Callus harvest for analysis
6-8	Transfer to PLB medium	MS + 2.0 mg/L BAP + 0.5 NAA	None
12	Mature PLBs	Same	PLB harvest for analysis
	Mother plant (field)	N/A	Pseudobulb tissue harvest

Callus was harvested for biochemical work at week six, when biomass was sufficient for triplicate extraction. PLBs were sampled at week twelve, by which point compact

green globular bodies averaging 3.2 mm diameter had formed.

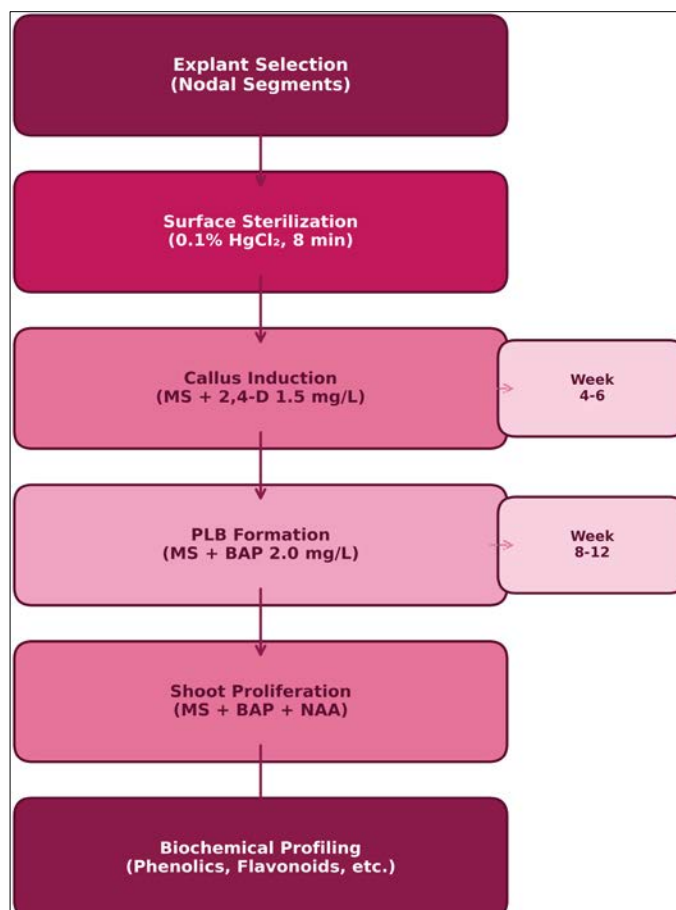


Fig 2: Schematic of the *in vitro* propagation pathway from explant to biochemical profiling for *D. crepidatum*, showing culture conditions and time frames

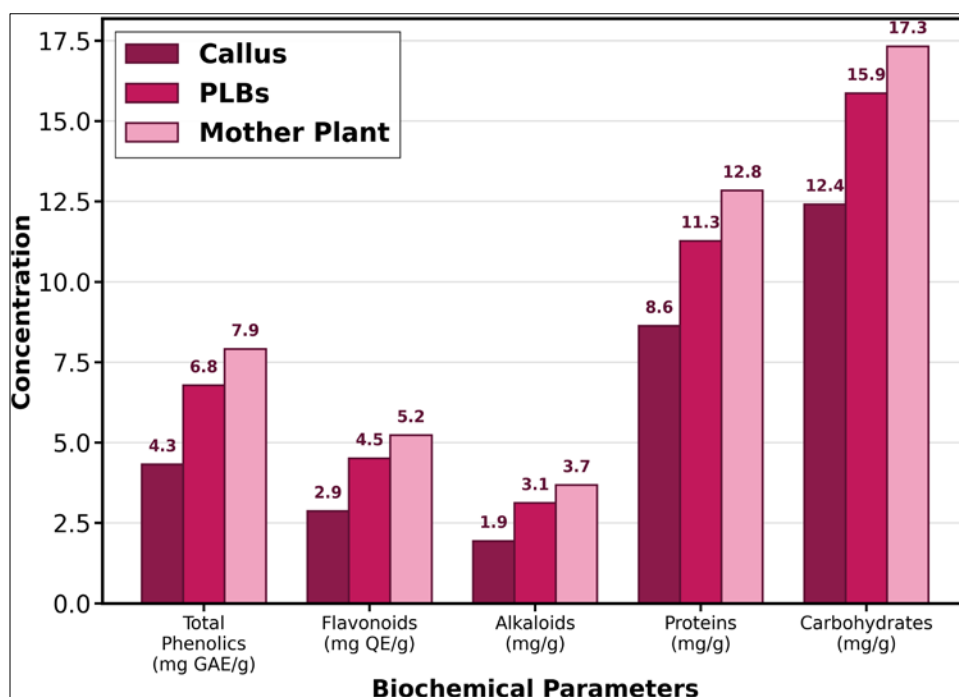


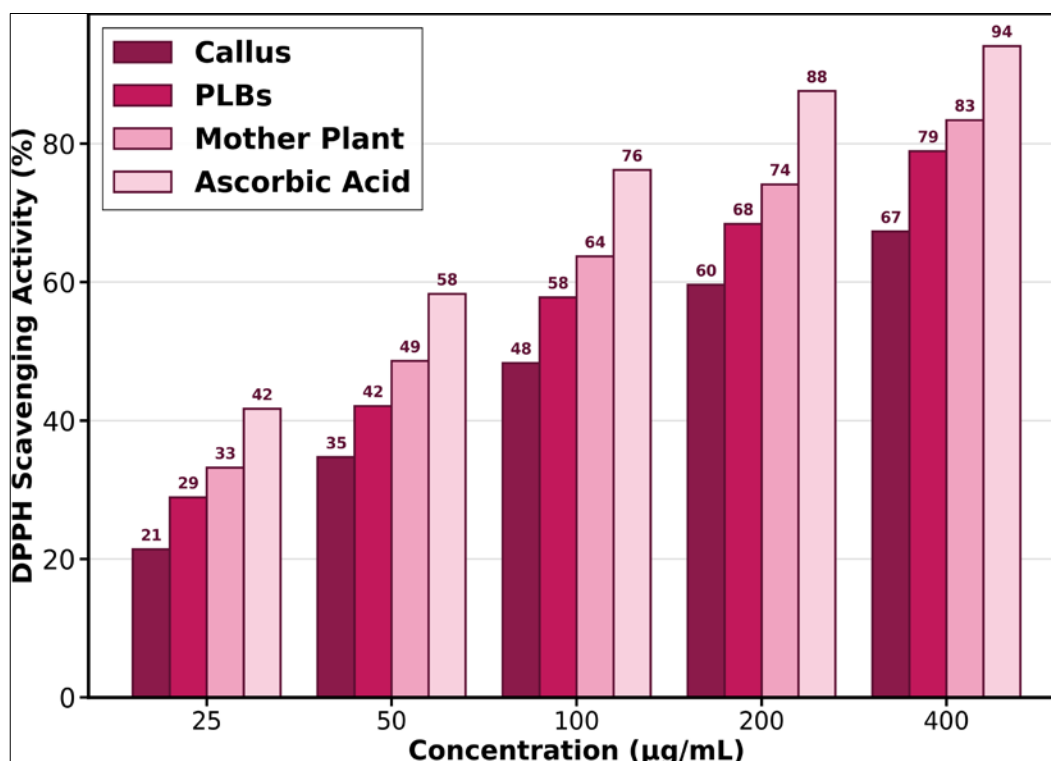
Fig 3: Concentrations of five major biochemical parameters in callus, PLBs, and mother plant tissue of *D. crepidatum*

Across all five parameters, callus extracts contained the lowest values, while mother plant tissue had the highest. The gap was most pronounced for total phenolics (callus

4.32 vs mother 7.91 mg GAE/g DW) and narrowest for total carbohydrates (12.41 vs 17.32 mg/g DW).

Table 2: DPPH radical scavenging activity (% inhibition) of methanolic extracts at five concentrations

Conc. (µg/mL)	Callus	PLBs	Mother Plant	Ascorbic Acid
25	21.4±1.8	28.9±2.1	33.2±2.4	41.7±1.6
50	34.7±2.3	42.1±2.7	48.6±3.1	58.3±2.0
100	48.3±3.1	57.8±2.9	63.7±2.8	76.2±1.9
200	59.6±2.7	68.4±3.2	74.1±2.5	87.6±1.4
400	67.3±3.4	78.9±2.8	83.4±2.1	94.1±1.1

**Fig 4:** Dose-dependent DPPH radical scavenging activity of callus, PLBs, mother plant, and ascorbic acid standard at five concentrations

Comprehensive interpretation

The biochemical gradient from callus through PLBs to mother plant tissue was consistent across every metabolite class measured. PLBs recovered between 77% and 91% of the mother plant's metabolite concentrations depending on the parameter, while callus reached only 50-72%. The dose-response curve for DPPH scavenging confirmed that antioxidant capacity tracked total phenolic and flavonoid content, as expected from the well-documented correlation between phenylpropanoid metabolites and radical-quenching ability. Ascorbic acid remained the strongest scavenger at every concentration, but the gap between PLB extract and the positive control narrowed at higher doses: at 400 µg/mL, PLBs reached 84% of ascorbic acid's activity. These patterns suggest that organogenesis progressively reactivates secondary metabolic pathways suppressed during dedifferentiation, and that PLB-stage biomass may offer a practical harvest point for bioactive compound recovery.

Discussion

Taken as a whole, the data show that *D. crepidatum* tissues cultured *in vitro* retain a measurable but reduced pool of secondary metabolites relative to field-grown plants, and that the degree of reduction depends on the developmental state of the culture. PLBs approximate mother plant chemistry far more closely than undifferentiated callus, a pattern that holds for phenolics, flavonoids, alkaloids, and antioxidant capacity alike.

This finding aligns with reports from other *Dendrobium* species. Zhao et al. [12] observed that polysaccharide content in *D. officinale* PLBs reached about 65% of greenhouse stem levels, a proportion similar to the phenolic recovery (86%) seen here for *D. crepidatum* PLBs. Teixeira da Silva and Ng [13] likewise reported that *D. nobile* PLBs accumulated dendrobine at concentrations approaching those of mature stems when coconut water was added to the medium. The agreement across species suggests a shared mechanism: as cells reorganise into meristematic centres during PLB formation, gene networks governing specialised metabolism—particularly the phenylpropanoid and terpenoid indole alkaloid pathways—are progressively reactivated [10, 14]. On the other hand, the callus-stage metabolite levels recorded here were lower than those reported by Kaur and Bhutani [9] for *Dendrobium fimbriatum* callus, where total phenolics exceeded 6 mg GAE/g DW at a similar 2,4-D concentration. One possible explanation is genotype sensitivity: *D. crepidatum* may channel more carbon toward primary growth under auxin stimulation, leaving fewer precursors for the shikimate pathway. Another factor could be light intensity: our cultures received 2,000 lux, whereas Kaur and Bhutani used 3,000 lux, and phenylalanine ammonia-lyase activity is known to be light-responsive [11]. From a broader standpoint, the results have two practical bearings. First, for conservation-driven mass propagation—as recently described by Bhowmik et al. [8]—it is reassuring that PLBs carry a biochemical profile close to wild-type tissue;

plants regenerated from PLBs are therefore likely to retain medicinal value once established in the field. Second, if the goal shifts toward using in-vitro biomass itself as a pharmaceutical feedstock, the data suggest harvesting at the PLB stage rather than the callus stage, because PLBs yield roughly 50% more phenolics and flavonoids per gram of dry tissue, with correspondingly stronger antioxidant activity. Optimisation of culture conditions increased light, elicitor treatments, or precursor feeding could potentially close the remaining gap between PLBs and mother plant tissue [15, 16].

Instrumentation and analytical equipment

All spectrophotometric measurements were carried out on a Shimadzu UV-1800 double-beam UV-Vis spectrophotometer fitted with 1 cm matched quartz cuvettes. Wavelengths used were 765 nm (Folin-Ciocalteu phenolics), 415 nm (aluminium chloride flavonoids), 470 nm (bromocresol green alkaloids), 595 nm (Bradford protein), 630 nm (anthrone carbohydrates), and 517 nm (DPPH scavenging). A Büchi R-210 rotary evaporator with a V-700 vacuum pump was used for solvent concentration. Analytical balances (Mettler Toledo ME204E, readability 0.1 mg) were employed for all weighing operations. Autoclaving was performed in a Hirayama HVE-50 vertical autoclave. The laminar flow cabinet (Esco Airstream) was UV-sterilised for 30 min before each culture session.

Conclusion

The main contribution of this research is a side-by-side biochemical comparison of callus, PLBs, and mother plant tissues of *D. crepidatum* a data set that was previously unavailable for this threatened medicinal orchid. Across six analytes, PLBs consistently recovered 77-91% of the mother plant's metabolite concentrations, while callus tissues retained only 50-72%. DPPH scavenging assays reinforced this hierarchy, with PLB extracts reaching 78.9% inhibition at 400 µg/mL compared to 67.3% for callus.

These figures carry significance on two fronts. For conservation programmes that rely on micropropagation to produce planting stock, the near-wild-type chemistry of PLBs provides confidence that regenerated plants will retain their medicinal properties once reintroduced. For pharmaceutical research, PLB-stage biomass represents a more productive starting material than callus for bioactive compound extraction, offering higher yields per unit of culture effort. Several questions remain open. Whether the metabolite recovery seen in PLBs persists through subsequent subculture passages has not been tested here and warrants longitudinal tracking. The influence of elicitor treatments methyl jasmonate, chitosan, or salicylic acid on pushing PLB metabolite levels closer to or beyond mother plant values is another promising avenue. And molecular confirmation, using techniques such as RAPD or ISSR markers, would verify that the genetic integrity underpinning these biochemical profiles remains intact through the culture process.

Acknowledgements

Contributions not qualifying for authorship

The authors acknowledge Ms. Le Thi Mai and Mr. Pham Quang Huy for technical help with spectrophotometric analyses and culture maintenance.

Funding Sources

This work was supported by internal research funds of the Vietnam National University, Hanoi. No external grants or commercial sponsorship were involved.

Institutional support

The Plant Biochemistry Laboratory and the Botany Herbarium of Vietnam National University, Hanoi, provided facilities, instrumentation, and access to authenticated plant material. The authors thank the nursery owner in Hoang Mai district for supplying source plants.

References

1. Hossain MM. Therapeutic orchids: traditional uses and recent advances an overview. *Fitoterapia*. 2011; 82(2):102-140.
2. Luo JP, Deng YY, Zha XQ. Mechanism of polysaccharides from *Dendrobium huoshanense* on streptozotocin-induced diabetic cataract. *Pharm Biol*. 2008; 46(4):243-249.
3. Chen XM, Wang FF, Wang YQ, Li XL, Wang AR, Wang CL, et al. Discrimination of the rare medicinal plant *Dendrobium officinale* based on naringenin, bibenzyl, and polysaccharides. *Sci China Life Sci*. 2012; 55(12):1092-1099.
4. Ng TB, Liu J, Wong JH, Ye X, Wing Sze SC, Tong Y, et al. Review of research on *Dendrobium*, a prized folk medicine. *Appl Microbiol Biotechnol*. 2012; 93(5):1795-1803.
5. Li XL, Xiao JJ, Zha XQ, Pan LH, Asghar MN, Luo JP. Structural identification and sulfated modification of an anti-glycation *Dendrobium huoshanense* polysaccharide. *Carbohydr Polym*. 2014; 106:247-254.
6. Pant B, Raskoti BB. Medicinal orchids of Nepal. Himalayan Research Institute; 2013. p. 1-104.
7. Fay MF. Orchid conservation: how can we meet the challenges in the twenty-first century? *Bot Stud*. 2018; 59:16.
8. Bhowmik TK, Rahman MM, Paul A, Paul T. Micropropagation of *Dendrobium crepidatum* Lindl. And Paxt.: an epiphytic medicinal orchid of Bangladesh. *Int J Biol Sci*. 2024; 6(1):7-13. DOI:10.33545/26649926.2024.v6.i1a.174.
9. Kaur S, Bhutani KK. *In vitro* conservation and aymbiotic propagation of *Dendrobium fimbriatum* a threatened orchid. *Plant Cell Tissue Organ Cult*. 2014; 118(1):1-11.
10. Murthy HN, Lee EJ, Paek KY. Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell Tissue Organ Cult*. 2014; 118(1):1-16.
11. Ramakrishna A, Ravishankar GA. Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behav*. 2011; 6(11):1720-1731.
12. Zhao P, Wu F, Feng FS, Wang WJ. Protocorm-like body (PLB) formation and plant regeneration from the

- callus culture of *Dendrobium candidum*. *in vitro* Cell Dev Biol Plant. 2008; 44(3):178-185.
13. Teixeira da Silva JA, Ng TB. The medicinal and pharmaceutical importance of *Dendrobium* species. *Appl Microbiol Biotechnol*. 2017; 101(6):2227-2239.
 14. Mohanty P, Das MC, Kumaria S, Tandon P. High-efficiency cryopreservation of the medicinal orchid *Dendrobium nobile* Lindl. *Plant Cell Tissue Organ Cult*. 2012; 109(3):415-421.
 15. Chugh S, Guha S, Rao IU. Micropropagation of orchids: a review on the potential of different explants. *Sci Hortic*. 2009; 122(4):507-520.
 16. Namdeo AG. Plant cell elicitation for production of secondary metabolites: a review. *Pharmacogn Rev*. 2007; 1(1):69-79.
 17. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant*. 1962; 15(3):473-497.
 18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965; 16(3):144-158.