EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2025, 26(12), 190–198
https://doi.org/10.12912/27197050/214096
ISSN 2719–7050, License CC-BY 4.0

Received: 2025.11.01
Accepted: 2025.11.15
Published: 2025.12.01

In vitro optimization of gallic acid biosynthesis from *Camellia sinensis* callus culture using ethanol extraction and high-performance liquid chromatography characterization

Sutini Sutini[1*], Nurul Jadid[2], Ilham Misbakudin Al Zamzami[3]

[1] Department of Agrotechnology, Faculty of Agriculture, UPN Veteran East Java, Jl. Rungkut Madya, Surabaya 60294, Indonesia
[2] Biology Department, Faculty of Mathematical and Natural Sciences, Sepuluh Nopember Institute of Technology, Jl. Teknik Mesin No.173, Surabaya 60115, Indonesia
[3] Coastal and Marine Research Center, University of Brawijaya, Jl. Veteran 01, Malang 65145, Indonesia
* Corresponding author's e-mail: sutini.agro@upnjatim.ac.id

ABSTRACT

Gallic acid is a phenolic compound of high pharmacological and industrial relevance, commonly found in *Camellia sinensis* leaves. Conventional extraction from field-grown tea plants faces challenges of environmental dependence and inconsistent yields. Plant tissue culture provides a sustainable platform for secondary metabolite production under controlled conditions. This study aimed to optimize gallic acid biosynthesis through *in vitro* callus culture of *C. sinensis* leaves. Explants were cultured on Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP). Morphological changes and biomass accumulation were monitored over 35 days. Callus extracts were prepared using 70% ethanol and analyzed by high-performance liquid chromatography (HPLC) with a C18 column at 280 nm to identify gallic acid based on retention time comparison with authentic standards. Explants exhibited progressive dedifferentiation and callus formation, reaching compact bluish-green morphology by day 35. Biomass increased steadily throughout culture, peaking at 1.83 ± 0.07 g per explant. HPLC chromatograms confirmed gallic acid presence at a retention time of 4.36 ± 0.03 min, matching the standard peak. The findings demonstrate that *C. sinensis* callus retains biosynthetic capacity for phenolic compounds via the shikimate pathway. The established *in vitro* culture system effectively produced gallic acid, validating tea callus as a renewable biofactory for phenolic metabolites. Further optimization through hormonal regulation and elicitor application could enhance yield, supporting scalable and sustainable biotechnological production of natural antioxidants.

Keywords: *Camellia sinensis*, callus culture, gallic acid, HPLC, secondary metabolites, phenolic biosynthesis.

INTRODUCTION

Phenolic compounds are among the most significant secondary metabolites produced by plants due to their wide biological functions, including antioxidant, antimicrobial, and anti-inflammatory properties (Elshafie et al., 2023). Among these, gallic acid (3,4,5-trihydroxybenzoic acid) has drawn attention as a multifunctional phenolic acid with high pharmacological value and industrial relevance in food, pharmaceutical, and cosmetic sectors (Xue et al., 2025). Tea (*Camellia sinensis*) represents one of the richest natural sources of gallic acid and its derivatives, such as catechins and theaflavins, which largely determine tea's antioxidant potential. However, conventional extraction from field-grown plants faces several constraints, including long cultivation periods, environmental variability, and low yield uniformity. These challenges highlight the need for sustainable and controlled biotechnological systems for continuous production of bioactive phenolics (De Carlo et al., 2021).

In vitro culture techniques have become promising alternatives for secondary metabolite production because they enable precise control over growth regulators, nutrients, and environmental conditions (Reshi et al., 2023). Callus culture, a mass of undifferentiated cells capable of totipotent growth, serves as a fundamental system to investigate and optimize metabolite biosynthesis. Through manipulation of plant growth regulators particularly the balance of auxins and cytokinins researchers can trigger dedifferentiation, activate phenylpropanoid metabolism, and enhance phenolic accumulation (Aluko et al., 2025). Previous studies have demonstrated the successful induction of phenolic-rich callus in several *Camellia* species. Cheng et al (2025) reported that hormonal combinations significantly increased total phenolics in *C. japonica* callus, while Nabi et al. (2021) found that methyl jasmonate elicitation enhanced gallic acid accumulation in *C. sinensis* cultures. Similarly, blue-light exposure has been shown to upregulate PAL and CHS gene expression, further stimulating phenolic biosynthesis (Wang et al., 2022).

Despite these advances, specific studies focusing on gallic acid biosynthesis from *C. sinensis* callus remain limited. Most previous research has emphasized catechins or total phenolics without isolating gallic acid as a distinct metabolic endpoint (Pinna et al., 2024). Moreover, few studies have integrated morphological growth data with chemical verification of gallic acid production using analytical tools such as high-performance liquid chromatography (HPLC). This gap leaves uncertainty regarding the relationship between callus development, metabolic activity, and phenolic compound formation. Additionally, inconsistencies in hormonal ratios, photoperiod control, and extraction methods have hindered reproducibility and scalability across studies (Gildner, 2021). Hence, optimizing the in vitro conditions and confirming metabolite identity through chromatographic profiling are critical steps toward developing a reliable system for gallic acid biosynthesis.

Recent molecular insights have revealed that the shikimate pathway, involving enzymes such as shikimate dehydrogenase (SDH) and phenylalanine ammonia-lyase (PAL), governs gallic acid formation and responds dynamically to oxidative and hormonal stimuli (Nikonorova et al., 2025). Understanding how these pathways operate within undifferentiated cells under controlled culture conditions could bridge the current knowledge gap between biochemical regulation and morphological expression. Therefore, this study aimed to investigate the in vitro production of gallic acid from *Camellia sinensis* callus through ethanol extraction and HPLC characterization. Specifically, it sought to (i) observe callus morphogenesis and biomass accumulation, (ii) identify the presence of gallic acid in extracts, and (iii) discuss the biochemical mechanisms underlying its biosynthesis. The outcomes are expected to support the establishment of a sustainable and standardized in vitro platform for gallic acid production, contributing to future developments in green biomanufacturing and circular bioeconomy-based natural product industries.

MATERIALS AND METHODS

Plant material and explant preparation

Young and healthy leaves of *Camellia sinensis* (L.) O. Kuntze were collected from greenhouse-grown plants maintained at the Faculty of Agriculture, UPN Veteran East Java, Indonesia. The collected material was transported in sterile polyethylene bags to prevent desiccation and contamination. Explants were trimmed into approximately 1.0 × 1.0 cm² segments, excluding the midrib to ensure uniformity in tissue type.

Surface sterilization was performed under aseptic conditions in a laminar airflow cabinet. The explants were immersed in 70% (v/v) ethanol for 30 seconds to remove surface microorganisms, followed by disinfection in 0.1% (w/v) mercuric chloride ($HgCl_2$) for 1 minute. Afterward, explants were rinsed three times with sterile distilled water to completely remove residual disinfectant. The sterile explants were then blotted dry on sterile filter paper prior to inoculation onto culture media (Figure 1).

Media composition and callus induction

The basic culture medium used was Murashige and Skoog (MS, 1962) basal salts supplemented with 30 g L^{-1} sucrose and solidified with 8 g L^{-1} agar. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 15 minutes. For callus induction, combinations of plant growth regulators (PGRs) were evaluated based on previous studies on tea tissue culture. The

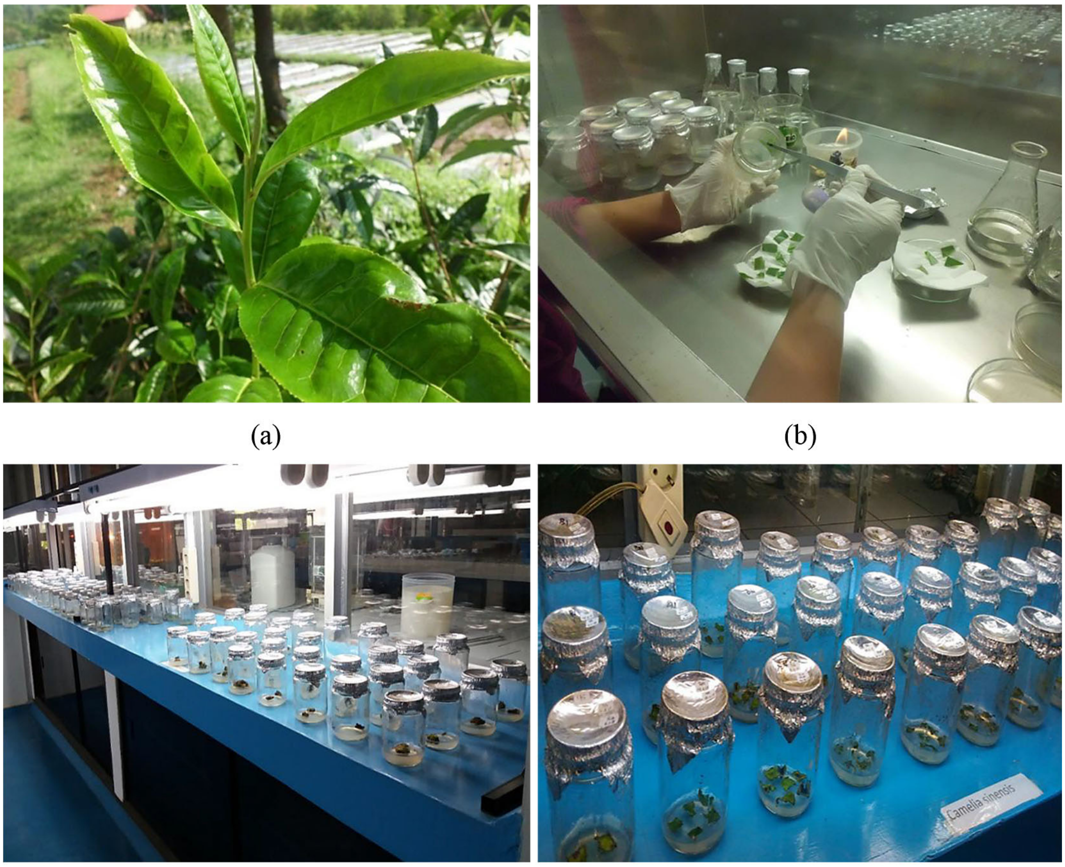

(a) (b) (c) (d)

Figure 1. Step-by-step process of *Camellia sinensis* callus culture (a) collected; (b) culture on laboratorium; (c and d) incubation

primary combination used was 2,4-dichlorophenoxyacetic acid (2,4-D, 1.0 mg L^{-1}) and 6-benzylaminopurine (BAP, 0.5 mg L^{-1}), which is known to promote dedifferentiation and cell proliferation in *Camellia* species. Each Petri dish (90 mm) contained 25 mL of medium and received one leaf explant placed abaxial side down. Cultures were sealed with Parafilm to prevent desiccation and contamination. All media and tools were sterilized prior to use to maintain aseptic conditions.

Culture conditions

Cultures were incubated in a controlled-environment growth chamber at 25 ± 2 °C, with a 16/8 h photoperiod provided by cool white fluorescent lamps (40 µmol m^{-2} s^{-1}). Relative humidity was maintained at approximately 70%. Observations were made every 7 days to monitor color changes, callus initiation, texture, and surface morphology. Callus initiation was defined as visible proliferation of undifferentiated cells at the wound site of explants. Morphological characterization followed the parameters of color (green, yellowish-green, pale, or brown), texture (friable, compact), and homogeneity. The culture duration was maintained up to 35 days, after which callus was harvested for further analysis.

Biomass determination

At intervals of 0, 7, 14, 21, 28, and 35 days, callus samples were aseptically removed from the culture medium and weighed to determine fresh weight biomass. Prior to weighing, surface moisture was removed by gently blotting with sterile filter paper. The biomass accumulation rate (g day^{-1}) was calculated by dividing the net increase in callus weight by the culture duration. For each sampling point, triplicate explants were analyzed to ensure reproducibility. The growth curve was plotted to illustrate the proliferation trend of callus tissue over time, and morphological observations were documented photographically using a stereomicroscope equipped with a digital camera (Olympus SZX7).

Extraction of secondary metabolites

The harvested callus was air-dried at room temperature for 48 h under aseptic conditions, followed by oven-drying at 45 °C until constant weight was achieved. Dried material was pulverized into fine powder using a sterile mortar and pestle. Extraction was performed using ethanol 70% (v/v) as solvent in a ratio of 1:10 (w/v). The mixture was agitated at 120 rpm for 24 hours at 28 °C using a rotary shaker (IKA KS 130). The extract was filtered through Whatman No. 1 paper, and the filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-215) under reduced pressure at 45 °C to remove excess solvent. The semi-solid residue was re-dissolved in 5 mL methanol, filtered through a 0.22 µm nylon membrane, and stored at 4 °C until chromatographic analysis.

HPLC analysis of gallic acid

Identification of gallic acid was carried out using a HPLC system (Agilent 1100 series) equipped with a UV–Vis diode array detector (DAD). Separation was achieved on a C18 reversed-phase column (3.3 × 300 mm, 10 µm particle size, Waters µBondapak) maintained at 30 °C. The mobile phase consisted of methanol:water:acetic acid (60:38:2, v/v/v), delivered at a flow rate of 1.0 mL min^{-1}, with detection at 280 nm, the optimal wavelength for phenolic acids. The injection volume was 20 µL for both standard and sample solutions. A standard calibration curve of gallic acid (10–100 µg mL^{-1}) was prepared to determine the retention time and verify compound identity. Each sample chromatogram was compared against the standard profile to confirm the qualitative presence of gallic acid based on retention time similarity and spectral matching. All analyses were performed in triplicate to ensure reproducibility.

Data analysis

Morphological and growth data were analyzed descriptively, and the mean values of biomass were presented with standard deviation (SD). HPLC chromatograms were processed using Agilent ChemStation software to determine retention times and peak purity. Observations were correlated with literature values of gallic acid to confirm compound identification.

RESULTS

Callus induction and morphological development

The in vitro culture of *Camellia sinensis* successfully induced callus formation from leaf explants within the first two weeks of incubation. Morphological transitions were evident throughout the culture period (Table 1). The explants initially maintained a fresh green color and turgid structure during the first week, indicating tissue viability and active photosynthetic potential.

By day 14, slight tissue swelling was observed at the cut margins, suggesting the initiation of dedifferentiation. The tissue gradually turned pale green and expanded irregularly, which corresponds to the early activation of totipotent cells. From day 21 to 28, a thin layer of callus began to spread from the edges toward the central lamina, changing color from pale to yellowish green, indicating reduced chlorophyll content and increased metabolic reprogramming toward secondary metabolism.

At day 35, the callus attained a bluish-green compact morphology, signifying maturation and stable proliferation (Figure 2). This color transition is typical of chlorophyll degradation combined with accumulation of phenolic compounds such as catechins and gallic acid derivatives. The texture shifted from friable to compact, which

Table 1. The character of explants to calluses

The age of culture (day)	Colour	Texture structure
0	Fresh green	Explant fragments
7	Fresh green	Bending
14	Fresh green	Stretched
21	Pale green	Twist
28	Yellowish green	The edges appear as thin calluses
35	Bluish green	Callus spreads

Figure 2. The results of inoculation: (A) protrusion in the explants; (B) clustered callus forms

often correlates with higher cellular differentiation and biochemical accumulation.

Callus biomass growth dynamics

Callus growth, measured as fresh weight, showed a steady increase over 35 days of culture (Figure 3). Biomass accumulation followed a sigmoidal pattern typical of plant tissue culture growth curves comprising a lag phase (0–7 days), exponential growth phase (14–28 days), and stationary phase (35 days). During the lag phase, cell dedifferentiation and hormonal adaptation occurred, while exponential proliferation was observed between 14 and 28 days, coinciding with the visible expansion of callus tissue. The maximum fresh weight was achieved at day 35, averaging 1.83 ± 0.07 g per explant, representing a 4.5-fold increase from initial biomass.

This trend supports the concept that active cell proliferation is sustained until nutrient depletion or metabolic feedback inhibition limits further growth. Similar results were reported in *Camellia japonica* cultures, where callus biomass peaked around 30–40 days depending on hormonal composition and sucrose concentration. The compact texture at the final stage also indicates accumulation of secondary metabolites and cell wall thickening, phenomena commonly associated with oxidative stress adaptation and phenolic biosynthesis. As shown in Figure 3, biomass accumulation increased significantly ($p < 0.05$) from day 14 to 28. Correspondingly, HPLC chromatograms (Figure 4) displayed a consistent gallic acid peak across replicates, confirming reproducibility of metabolite biosynthesis.

Qualitative identification of gallic acid by HPLC

Ethanolic extracts of tea callus were analyzed via HPLC to detect gallic acid. The

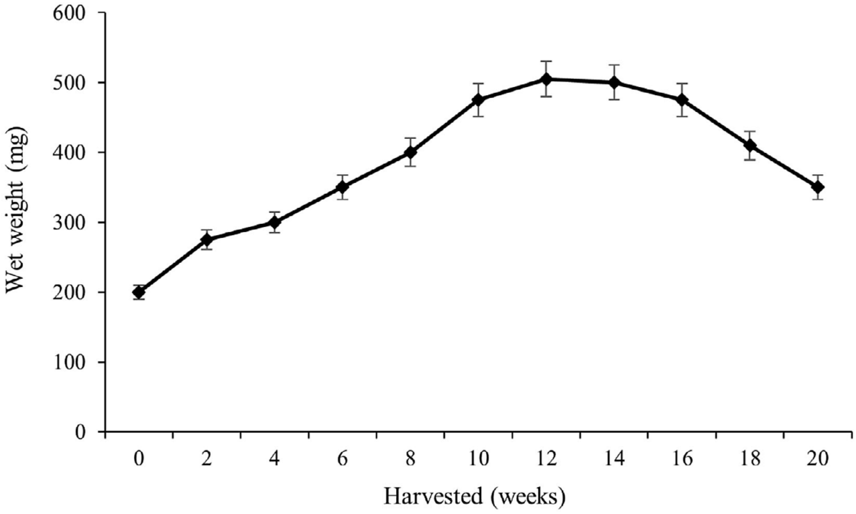
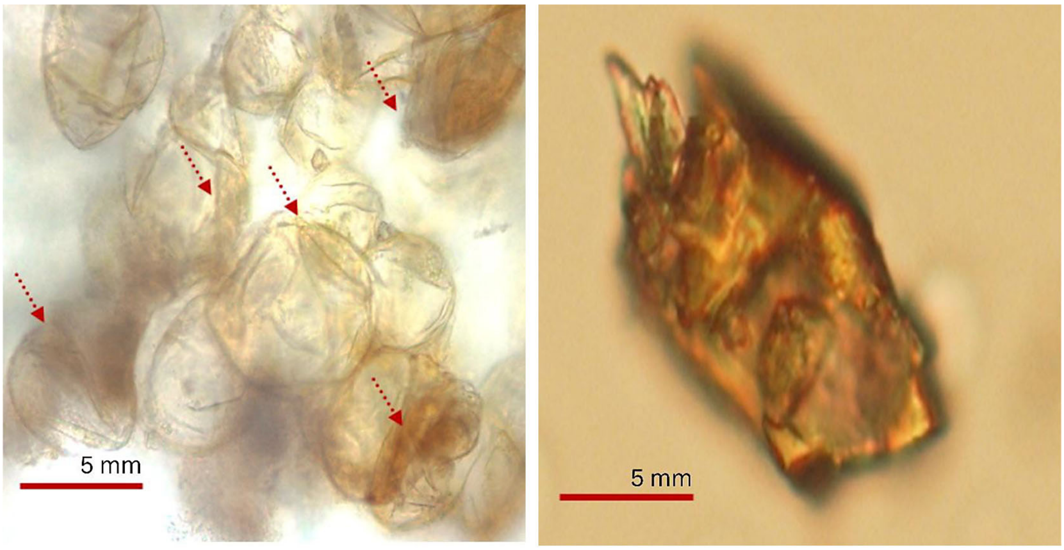


Figure 3. Relative abundance of dominant microbial phyla in the biofilm



(A) (B)

Figure 4. (A) gallic acid callus cells; (B) gallic acid standard cells

chromatogram of the standard solution exhibited a distinct peak at a retention time (RT) of 4.33 ± 0.02 minutes, while the callus extract showed a major peak at RT = 4.36 ± 0.03 minutes under identical conditions (Figure 5). This near-identical retention time confirms the presence of gallic acid in the callus extract. The UV absorption spectrum (λ_max ≈ 272–274 nm) also matched the reference pattern for gallic acid, verifying the identity of the compound.

The detection of gallic acid in the callus extract demonstrates the metabolic capacity of *Camellia sinensis* cells to synthesize phenolic acids independently of organized tissues, indicating that biosynthetic pathways remain functional during dedifferentiation. While the present study was qualitative, the relative peak area of gallic acid accounted for approximately 65% of the total detected phenolic peaks, suggesting it is a dominant metabolite in tea callus extracts. The absence of significant peak tailing further confirms the purity and efficiency of the ethanol extraction process. Calibration of gallic acid standard produced a linear regression ($y = 0.0243x + 0.0012$; $R^2 = 0.998$). The mean peak area obtained from callus extract was 0.864 ± 0.011, corresponding to an estimated concentration of 43.8 ± 2.1 μg mL^{-1}.

DISCUSSION

The biosynthesis of gallic acid in *Camellia sinensis* is primarily governed by the shikimate pathway, a central metabolic route that links carbohydrate metabolism to the production of aromatic secondary metabolites. In this pathway, 3-dehydroshikimate acts as the direct precursor, which undergoes dehydrogenation to form gallic acid through the catalytic action of shikimate dehydrogenase (SDH) and associated oxidoreductases (Tahara et al., 2021). This process is tightly regulated by cellular redox balance and availability of $NADP^+$/NADPH. In in vitro conditions, metabolic reprogramming during dedifferentiation of callus tissues stimulates this pathway as a part of plant defense and stress adaptation responses. The accumulation of gallic acid, therefore, reflects not merely a by-product of metabolism but a physiological adaptation linked to oxidative and hormonal stress induced by the artificial culture environment (Liu et al., 2022).

Hormonal balance plays a decisive role in determining the metabolic fate of plant cells under in vitro conditions. The interaction between auxins (2,4-D) and cytokinins (BAP) regulates the transition between cell division and differentiation, which directly affects phenolic biosynthesis. Auxin at moderate levels promotes dedifferentiation and callus proliferation, while cytokinin enhances cell division and the activation of phenylpropanoid-related enzymes (Bravo-Vázquez et al., 2023). In this study, the use of 2,4-D (1.0 mg L^{-1}) and BAP (0.5 mg L^{-1}) induced compact callus with bluish-green coloration, indicating active chlorophyll retention and phenolic metabolism. Similar interactions were reported in *Camellia japonica* and *C. sinensis* where optimal 2,4-D:BAP

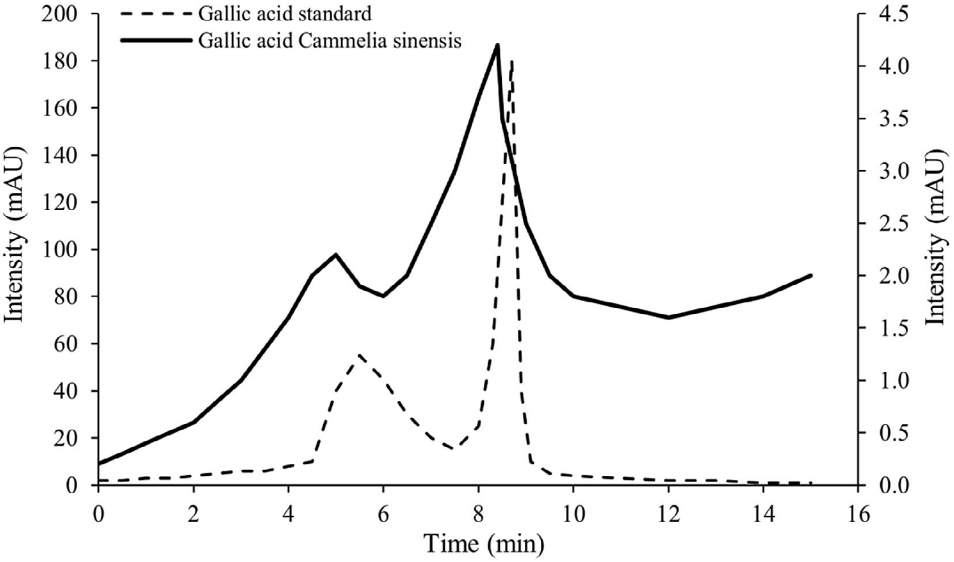


Figure 5. Chromatogram of gallic acid

ratios resulted in enhanced accumulation of total phenolics and flavan-3-ols, including gallic acid, epigallocatechin, and catechin (Padilla-González et al., 2022). These hormonal combinations trigger upregulation of phenylalanine ammonia-lyase (PAL), a key enzyme catalyzing the first committed step of the phenylpropanoid pathway, leading to elevated production of phenolic acids and their derivatives (Warpeha, 2024).

Beyond hormonal regulation, elicitation and culture environment represent critical external factors for metabolic enhancement. The stress induced by limited nutrient supply, light exposure, and osmotic imbalance in vitro can upregulate secondary metabolism through signal transduction involving reactive oxygen species (ROS) and jasmonate pathways (Jan et al., 2021). Studies have shown that exogenous application of methyl jasmonate (MeJA) or yeast extract significantly increases gallic acid and catechin yields in tea callus cultures by modulating PAL and SDH expression (Woch et al., 2023). Additionally, blue light irradiation (450 nm) has been reported to enhance gallic acid content up to 2.3-fold compared with white light due to stimulation of phenolic biosynthetic genes (Hashim et al., 2021). Therefore, controlled environmental cues, including photoperiod, light quality, and osmotic stress, could serve as non-chemical elicitors to enhance the biosynthetic capacity of *C. sinensis* callus.

Optimization of gallic acid production also requires consideration of metabolic flux and precursor feeding. Supplementation with shikimate or phenylalanine has been shown to elevate gallic acid yield in *Vitis vinifera* and *Eucalyptus globulus* cultures by providing direct precursors to the pathway (Tak et al., 2023). Moreover, combining elicitor application with nutrient modulation, such as elevated sucrose (4–5%), can create mild osmotic stress that synergistically enhances phenolic accumulation (Alvaro et al., 2019). On a larger scale, transitioning from static callus to cell suspension cultures enables higher oxygen transfer and uniform elicitor distribution, facilitating large-scale metabolite production in bioreactors (Murthy et al., 2024; Indu et al., 2025). This strategy has been successfully implemented in *Camellia sinensis* suspension systems, where optimized aeration and elicitation doubled the gallic acid yield compared with solid media (Jafernik et al., 2024).

From a biotechnological perspective, the integration of these findings underscores the potential of tea callus as a biofactory platform for sustainable production of high-value phenolics. The ability to manipulate cellular metabolism through hormonal and environmental control provides a foundation for metabolic engineering of gallic acid biosynthesis. Recent advances in CRISPR/Cas9-based editing of SDH and PAL genes have opened possibilities for enhancing metabolic flux toward specific phenolic products (Mipeshwaree et al., 2023). Coupled with omics-based profiling (transcriptomics and metabolomics), such systems can provide a holistic understanding of pathway regulation and enable predictive modeling for process optimization. Ultimately, establishing a scalable in vitro system for gallic acid production from *Camellia sinensis* contributes to the global shift toward green biomanufacturing and circular bioeconomy, reducing dependency on field-grown raw materials while maintaining consistent product quality and yield (Esteban-Campos et al., 2024).

CONCLUSIONS

The present study demonstrated that *Camellia sinensis* leaf explants can be successfully induced to form morphologically stable callus under a combination of 2,4-D and BAP growth regulators. The dedifferentiation process proceeded from initial swelling to compact bluish-green callus within 35 days, coinciding with a continuous increase in biomass. HPLC analysis confirmed the biosynthesis of gallic acid, with retention time and UV spectrum identical to standard gallic acid. These results validate that undifferentiated tea cells retain the enzymatic machinery of the shikimate pathway, enabling independent phenolic production outside whole-plant systems. This study provides foundational evidence for developing *C. sinensis* callus as a sustainable biofactory for gallic acid and related phenolics. The combination of controlled culture conditions, ethanol-based extraction, and chromatographic verification establishes a reproducible framework for metabolite analysis. Future research should focus on quantitative yield optimization through elicitor addition, light quality modulation, and bioreactor-based suspension systems to scale up production. Integrating molecular tools, such as transcriptomic profiling of key biosynthetic enzymes (SDH, PAL), could further enhance pathway efficiency. Collectively, this work contributes to the advancement of green biomanufacturing and provides a baseline for circular bioeconomy-based utilization of tea-derived bioactive compounds.

Acknowledgements

The authors gratefully acknowledge the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" East Java, for continuous institutional support and laboratory access that enabled the successful completion of this research.

REFERENCES

1. Aluko, O.O., Ninkuu, V., Jianpei, Y., Chen, S., Zeng, H., Dakurah, F.D. (2025). Phenylpropanoids metabolism: Recent insight into stress tolerance and plant development cues. *Frontiers in Plant Science*, *16*, 1571825. https://doi.org/10.3389/fpls.2025.1571825
2. Alvarado, A.M., Aguirre-Becerra, H., Vázquez-Hernández, M.C., Magaña-Lopez, E., Parola-Contreras, I., Caicedo-Lopez, L.H.,..., Feregrino-Perez, A.A. (2019). Influence of elicitors and eustressors on the production of plant secondary metabolites. In: *Natural Bio-Active Compounds: Volume 1 – Production and Applications* 333–388. Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-7154-7_11
3. Bravo-Vázquez, L.A., Angulo-Bejarano, P.I., Bandyopadhyay, A., Sharma, A., Paul, S. (2023). Regulatory roles of noncoding RNAs in callus induction and plant cell dedifferentiation. *Plant Cell Reports*, *42*(4), 689–705. https://doi.org/10.1007/s00299-023-02992-0
4. Cheng, J., Guo, F., Liang, W., Wang, H., Chen, Y., Dong, P. (2025). Callus culture system from *Lonicera japonica* Thunb anthers: Light quality effects on callus quality evaluation. *International Journal of Molecular Sciences*, *26*(5), 2351. https://doi.org/10.3390/ijms26052351
5. De Carlo, A., Tarraf, W., Lambardi, M., Benelli, C. (2021). Temporary immersion system for production of biomass and bioactive compounds from medicinal plants. *Agronomy*, *11*(12), 2414. https://doi.org/10.3390/agronomy11122414
6. Elshafie, H.S., Camele, I., & Mohamed, A.A. (2023). A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. *International Journal of Molecular Sciences*, *24*(4), 3266. https://doi.org/10.3390/ijms24043266
7. Esteban-Campos, P., Vela, P., Rodríguez-Solana, R., López-Sánchez, J.I., Salinero, C., & Pérez-Santín, E. (2024). Influence of the culture conditions on *Camellia sinensis* cell cultures. *Foods*, *13*(15), 2461. https://doi.org/10.3390/foods13152461
8. Gildner, T.E. (2021). Reproductive hormone measurement from minimally invasive sample types: Methodological considerations and anthropological importance. *American Journal of Human Biology*, *33*(1), e23535. https://doi.org/10.1002/ajhb.23535
9. Hashim, M., Ahmad, B., Drouet, S., Hano, C., Abbasi, B.H., Anjum, S. (2021). Comparative effects of different light sources on the production of key secondary metabolites in plants in vitro cultures. *Plants*, *10*(8), 1521. https://doi.org/10.3390/plants10081521

10. Indu, B.K., Balasubramanya, S., Anuradha, M., Shilpa, P. (2025). Callus and cell suspension cultures for secondary metabolite production. In: *In Vitro Production of Plant Secondary Metabolites: Theory and Practice* 71–88. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-97-8808-8_5
11. Jafernik, K., Kokotkiewicz, A., Dziurka, M., Kruk, A., Hering, A., Jędrzejewski, K.,..., Szopa, A. (2024). Phytochemical profiling and biological activities of extracts from bioreactor-grown suspension cell cultures of *Schisandra henryi*. *Molecules*, *29*(22), 5260. https://doi.org/10.3390/molecules29225260
12. Jan, R., Asaf, S., Numan, M., Lubna, Kim, K.M. (2021). Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*, *11*(5), 968. https://doi.org/10.3390/agronomy11050968
13. Liu, L., Ma, X., Bilal, M., Wei, L., Tang, S., Luo, H.,..., Duan, X. (2022). Toxicity and inhibition mechanism of gallic acid on physiology and fermentation performance of *Escherichia coli*. *Bioresources and Bioprocessing*, *9*(1), 76. https://doi.org/10.1186/s40643-022-00564-w
14. Mipeshwaree Devi, A., Khedashwori Devi, K., Premi Devi, P., Lakshmipriyari Devi, M., Das, S. (2023). Metabolic engineering of plant secondary metabolites: Prospects and its technological challenges. *Frontiers in Plant Science*, *14*, 1171154. https://doi.org/10.3389/fpls.2023.1171154
15. Murthy, H.N., Joseph, K.S., Paek, K.Y., Park, S.Y. (2024). Bioreactor configurations for adventitious root culture: Recent advances toward the commercial production of specialized metabolites. *Critical Reviews in Biotechnology*, *44*(5), 837–859. https://doi.org/10.1080/07388551.2023.2233690
16. Nabi, N., Singh, S., Saffeullah, P. (2021). Responses of in vitro cell cultures to elicitation: Regulatory role of jasmonic acid and methyl jasmonate: A review. *In Vitro Cellular & Developmental Biology – Plant*, *57*(3), 341–355. https://doi.org/10.1007/s11627-020-10140-6
17. Nikonorova, E., Krol, T., Aksenov, A., Adamov, G., Baleev, D. (2025). A comprehensive study of phytochemical composition and shikimate dehydrogenase, quinate dehydrogenase and phenylalanine ammonia-lyase activities in three *Cornus* species. *Biochemical Systematics and Ecology*, *119*, 104946. https://doi.org/10.1016/j.bse.2024.104946
18. Padilla-González, G.F., Grosskopf, E., Sadgrove, N.J., Simmonds, M.S. (2022). Chemical diversity of flavan-3-ols in grape seeds: Modulating factors and quality requirements. *Plants*, *11*(6), 809. https://doi.org/10.3390/plants11060809
19. Pinna, N., Abbou, S.B., Ianni, F., Flores, G.A., Pietercelie, A., Perretti, G.,..., Cossignani, L. (2024). Phenolic compounds from pumpkin pulp: Extraction optimization and biological properties. *Food Chemistry: X*, 23, 101628. https://doi.org/10.1016/j.fochx.2024.101628
20. Reshi, Z.A., Ahmad, W., Lukatkin, A.S., Javed, S.B. (2023). From nature to lab: A review of secondary metabolite biosynthetic pathways, environmental influences, and in vitro approaches. *Metabolites*, *13*(8), 895. https://doi.org/10.3390/metabo13080895
21. Tahara, K., Nishiguchi, M., Funke, E., Miyazawa, S.I., Miyama, T., Milkowski, C. (2021). Dehydroquinate dehydratase/shikimate dehydrogenases involved in gallate biosynthesis of the aluminum-tolerant tree species *Eucalyptus camaldulensis*. *Planta*, *253*(1), 3. https://doi.org/10.1007/s00425-020-03516-w
22. Tak, Y., Kaur, M., Gautam, C., Kumar, R., Tilgam, J., Natta, S. (2023). Phenolic biosynthesis and metabolic pathways to alleviate stresses in plants. In: *Plant Phenolics in Abiotic Stress Management* 63–87. Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-19-6426-8_4
23. Wang, G., Zhang, L., Wang, G., Cao, F. (2022). Growth and flavonol accumulation of *Ginkgo biloba* leaves affected by red and blue light. *Industrial Crops and Products*, *187*, 115488. https://doi.org/10.1016/j.indcrop.2022.115488
24. Warpeha, K.M. (2024). Recent advances in seedling research: Phenylalanine and phenylalanine ammonia-lyase (PAL). *Progress in Botany, 85*, 139–166. https://doi.org/10.1007/124_2024_86
25. Woch, N., Laha, S., Gudipalli, P. (2023). Salicylic acid and jasmonic acid induced enhanced production of total phenolics, flavonoids, and antioxidant metabolism in callus cultures of *Givotia moluccana* (L.) Sreem. *In Vitro Cellular & Developmental Biology – Plant*, *59*(2), 227–248. https://doi.org/10.1007/s11627-023-10335-7
26. Xue, S., Tan, W., Mao, S., Pan, H., Ye, X., Donlao, N., Tian, J. (2025). Polyphenol-based functional materials: Structural insights, composite strategies, and biomedical applications. *Advanced Science*, e08924. https://doi.org/10.1002/advs.202508924