Plant Sci. Arc.
Plant Science Archives
An International Journal of Plant Biology

Original Research Article
ISSN 2518-6965
Journal Homepage: www.plantarc.com

Optimizing callus induction, indirect organogenesis and phytochemical profiling of *Alpinia galanga*

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Citation: Suprava Sahoo, Jyotirmayee Lenka, and Basudeba Kar (2025). Optimizing callus induction, indirect organogenesis and phytochemical profiling of *Alpinia galanga*. *Plant Science Archives.* **DOI: https://doi.org/10.51470/PSA.2025.10.4.13**

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Received 17 July 2025 | Revised 13 August 2025 | Accepted 14 September 2025 | Available Online 13 October 2025

ABSTRACT

Alpinia galanga, a medicinally important rhizomatous plant, is valued for its bioactive compounds and essential oils, which exhibit diverse pharmacological activities. The present study evaluated the in vitro culture establishment, phytochemical composition, and antioxidant potential of A. galanga. Callus induction from explants was strongly influenced by growth regulator treatments. Treatment with 2,4-D at 2 mg L^{-1} yielded the highest embryogenic callus induction (48.63%), while higher concentrations were inhibitory. The combination of 2 mg L^{-1} 2,4-D with 0.3 mg L^{-1} TDZ significantly enhanced induction (76.84%), demonstrating synergistic effects. Shoot regeneration from embryogenic cell suspensions was maximized with 2 mg L^{-1} BA alone, achieving 75.24% regeneration and 11.46 shoots per callus, whereas BA–kinetin combinations reduced efficiency. GC-MS analysis of conventionally propagated and callus-derived leaf and rhizome oils identified 45 volatile compounds, predominantly monoterpenes, sesquiterpenes, and phenylpropanoids. Major constituents such as eucalyptol, β-pinene, and camphor were retained, while guaiol and fenchyl acetate showed increased accumulation in callus-derived oils. Callus induction also enhanced total phenolic (TPC: 84.38 ± 1.03 mg GAE/g) and flavonoid contents (TFC: 75.33 ± 1.17 mg QE/g) compared to conventional propagation. Antioxidant activity assessed via DPPH assay revealed strong radical scavenging potential, with callus-induced rhizome oil exhibiting the lowest IC_{50} (14 µg/ml), surpassing ascorbic acid (18 µg/ml). Overall, in vitro culture effectively preserves phytochemical profiles while enhancing bioactive compound accumulation and antioxidant capacity in A. galanga, highlighting its potential for sustainable production of natural antioxidants.

Keywords: *Alpinia galanga, callus, micropropagation, essential oils, antioxidant activity, TPC, TFC.*

Introduction

Alpinia galanga (L.) Willd., commonly known as greater galangal, is a perennial rhizomatous herb of the family Zingiberaceae. Native to India and Southeast Asia, it is widely distributed throughout tropical and subtropical regions of the world [1]. The plant holds immense value in traditional systems of medicine such as Ayurveda, Unani, Siddha, and folk practices, where its rhizomes are prescribed for ailments ranging from fever, bronchitis, chest pain, skin diseases and rheumatism, to kidney stones, ulcers, and gastrointestinal disorders [2][1]. Apart from its medicinal use, it is also employed as a culinary spice, flavoring agent, and as a raw material in perfumery and aromatherapy due to its characteristic earthy–mint aroma [3].

The rhizome is the most economically important part, containing a complex mixture of bioactive phenylpropanoids, flavonoids, tannins, terpenes and essential oils (EOs). Compounds such as 1′S-1′-acetoxychavicol acetate (ACA), acetoxyeugenol acetate, kaempferol derivatives, galangin, and 1,8-cineole have been reported as key phytochemicals contributing to its therapeutic potential [2][1]. These bioactive constituents demonstrate a wide range of pharmacological activities, such as antioxidant, antimicrobial (antibacterial, antifungal, antiviral, and antiprotozoal), anti-inflammatory, antidiabetic, hypolipidemic, immunomodulatory, antiplatelet, anticancer, and anti-HIV effects.
[4].

Propagation is usually vegetative, through rhizome division, though this method is limited by low multiplication rate, susceptibility to rhizome rot pathogens, and heavy demand for planting material [5][6]. Consequently, the species faces threats of overexploitation and has been listed among the 195 Red Listed Medicinal Plants by FRLHT (Foundation for Revitalization of Local Health Traditions, Bengaluru, 1997) [4]. Given the increasing pharmaceutical and commercial demand, biotechnological interventions such as *in vitro* propagation, somatic embryogenesis, suspension cultures, and genetic transformation offer promising strategies for conservation, large-scale multiplication, and metabolic enhancement [4][6]. Plant tissue culture techniques provide numerous advantages, such as being cost-effective, enabling rapid large-scale propagation, and facilitating the production of valuable natural compounds.

Furthermore, plant regeneration through embryogenic callus culture and protoplast transformation can facilitate both crop improvement and conservation, ensuring the availability of genetically superior, and high-yielding planting material. Extensive research has been done for in vitro clonal propagation using the auxiliary bud explant of *Alpinia galanga* [7][8]. However, a significant gap exists in the literature concerning the comparative phytochemical profiling between naturally grown and callus-induced *A. galanga*. Therefore, the present study aims to establish a reliable protocol for induction of callus and subsequent plant regeneration in *A. galanga*, using shoot base explants, with a view to contributing towards its conservation and pharmaceutical utility. In addition, the investigation also encompasses the assessment of antioxidant potential, GC–MS–based phytochemical profiling, and quantitative estimation of total phenolic content (TPC) and total flavonoid

content (TFC), thereby providing an integrated understanding of both the biotechnological and biochemical dimensions of this important medicinal species.

Materials and Methods

Plant material

Rhizomes of *A. galanga* were sourced from the Khurda district of Odisha and authenticated by a taxonomist. A voucher specimen (No. 10688) has been preserved in the institutional greenhouse for future reference. The authenticated plant material was subsequently cultivated in the medicinal plant garden of the Siksha 'O' Anusandhan University, Bhubaneswar.

Establishment of callus culture:

Young buds (0.5–1.5 cm) from healthy *A. galanga* plants were aseptically excised and cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) following a modified protocol of Parida et al. [8] for regeneration studies. About one-month-old *in vitro*–grown shoot base explants were used for callus induction. The explants were transferred to MS agar medium supplemented with varying concentrations of 2,4-D (1–3 mg/L), either alone or in combination with other growth regulators BA (2–3 mg/L), Kn (0.5–1.0 mg/L), or TDZ (0.3–0.6 mg/L). Cultures were incubated in darkness at 26 ± 1 °C, and the most responsive treatments involving optimal regulator and nitrogen combinations were selected. Embryogenic calli were subcultured at 21-day intervals, with 15 replicates maintained per treatment for statistical analysis.

Plant regeneration

Callus aggregates were shifted to a solid MS medium to promote further proliferation. For shoot induction, these aggregates were cultured on MS agar medium supplemented with different concentrations of BA, Kn, and NAA. Cultures were maintained at 26 ± 1 °C under fluorescent illumination (~3000 lux) with a 16 h light/8 h dark cycle. Regeneration efficiency was evaluated based on the percentage of explants forming shoots and the average number of shoots generated per embryogenic aggregate.

Plant hardening and oil extraction

Ninety-day-old in vitro–derived plantlets with well-developed shoots and roots were transferred to pots containing a soil:cow dung:sand mixture (1:1:1, v/v) for acclimatization under greenhouse conditions for 30 days. After successful hardening, the plants were moved to field conditions and grown to full maturity.

At maturity, 100 g of rhizomes from field-grown plants were hydro-distilled for 4 hours in a Clevenger-type apparatus to obtain essential oil (EO). The extracted oil was dried over anhydrous sodium sulfate, stored in amber vials at 4 °C, and quantified based on the volume of oil per gram of fresh rhizome weight.

GC-MS Analysis

EOs from field-grown and *in vitro*–derived rhizomes were analyzed by GC–MS under identical conditions using a Clarus 580 Gas Chromatograph coupled with an SQ8S mass detector (PerkinElmer, USA). Helium was used as the carrier gas (1.0 mL/min), and a 0.1 µL oil sample was injected in split mode onto an Elite-5 capillary column (30 m × 0.25 mm, 0.25 µm film). The oven temperature was programmed from 50°C (1 min hold) to 230°C at 5°C/min, then to 260°C at 15°C/min (1 min hold), giving a total runtime of 45 min. Injector, transfer line, and ion source temperatures were kept at 260°C. Mass spectra were recorded in EI mode (70 eV) over m/z 50–600. Compounds were identified by comparing their mass spectra and retention indices with the NIST 11 library, Adams database [9], and literature values. Retention indices were determined using a homologous series of n-alkanes analyzed under the same chromatographic conditions.

Determination of Total Phenolic and Flavonoid Content

The total phenolic (TPC) and flavonoid (TFC) contents of EOs from field-grown and *in vitro*–cultured *A. galanga* rhizomes were determined using modified colorimetric methods [10]. TPC was estimated by the Folin–Ciocalteu assay with gallic acid as the standard. Diluted EO samples (250 µL) were mixed with Folin–Ciocalteu reagent and sodium carbonate solution, incubated for 90 min at room temperature, and absorbance was read at 760 nm. Results were expressed as mg gallic acid equivalents (GAE) per g of extract.

TFC was measured using the aluminum chloride method [10]. EO samples were reacted with 2% $AlCl_3$ solution in ethanol, incubated for 1 h in the dark, and absorbance was taken at 420 nm. Values were expressed as mg quercetin equivalents (QE) per g of extract. All measurements were performed in triplicate, and data are presented as mean ± standard deviation.

Evaluation of antioxidant activity

The antioxidant activity of EOs from field-grown and *in vitro*–derived *A. galanga* rhizomes was evaluated using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay as described by Lenka et al. [10] with minor modifications.

EO samples were diluted in methanol to different concentrations (1–100 µg/mL) and mixed with an equal volume of 0.1 mM DPPH solution. After incubation in the dark for 30 min at room temperature, absorbance was recorded at 517 nm against methanol as blank. Ascorbic acid served as the reference standard, and each test was performed in triplicate.

The percentage of DPPH radical scavenging activity was calculated using the following equation: % Inhibition = $[(A_{control}-A_{test})/A_{control}] \times 100$

where $A_{control}$ is the absorbance of the DPPH solution and A_{test} is that of the sample. Antioxidant strength was expressed as IC_{50}, representing the concentration required to scavenge 50% of DPPH radicals.

Result

In vitro culture establishment by indirect regeneration:

Callus induction from *A. galanga* explants was strongly influenced by the type and concentration of growth regulators used in the MS medium. Among the treatments containing only 2,4-D, the optimal embryogenic callus formation was observed at 2 mg L^{-1}, achieving a response rate of 48.63%, while 1 mg L^{-1} yielded a slightly lower rate of 39.78%. A further increase to 3 mg L^{-1} led to a notable decline in induction efficiency (20.65%), suggesting that elevated concentrations of 2,4-D negatively affect embryogenesis. When 2,4-D was combined with TDZ, callus induction improved markedly compared to treatments with 2,4-D alone (Table 1). The best response (76.84%) was achieved with 2 mg L^{-1} 2,4-D and 0.3 mg L^{-1} TDZ, followed by 67.52% for the combination of 2 mg L^{-1} 2,4-D and 0.2 mg L^{-1} TDZ. However, increasing the TDZ concentration to 0.5 mg L^{-1} led to a reduction in callus formation efficiency (49.57%). At higher levels of 2,4-D (3 mg L^{-1}), supplementation with TDZ

produced comparatively lower responses, ranging from 37.38% (with 0.2 mg L^{-1} TDZ) to 49.95% (with 0.5 mg L^{-1} TDZ). Collectively, these results suggest that a moderate concentration of 2,4-D (2 mg L^{-1}) combined with a low concentration of TDZ (0.3 mg L^{-1}) is the most favorable condition for efficient embryogenic callus induction in *A. galanga*.

Influence of growth regulators on shoot regeneration in *A. galanga*

The effect of various plant growth regulators on shoot regeneration from embryogenic cell suspension cultures of *A. galanga* was assessed (Table 2). The highest regeneration rate (75.24 ± 0.72%) and maximum shoot number (11.46 ± 0.12 per callus) were achieved on MS medium containing 2 mg L^{-1} BA. Increasing the concentration of BA to 3 mg L^{-1} reduced regeneration percentage to 58.63 ± 0.55% and shoots per callus to 8.62 ± 0.33. Combinations of BA with kinetin (Kn) were less effective; 2 mg L^{-1} BA + 0.5 mg L^{-1} Kn yielded 50.67 ± 0.34% regeneration with 6.29 ± 0.22 shoots per callus, while 2 mg L^{-1} BA + 1.0 mg L^{-1} Kn further decreased regeneration to 37.28 ± 0.25% and shoots per callus to 4.83 ± 0.34 **(Table 2)**. These results indicate that BA alone at 2 mg L^{-1} is most effective for plantlet regeneration from embryogenic cell suspensions in *A. galanga*, and addition of kinetin or higher BA concentrations reduces regeneration efficiency.

GC-MS analysis

GC-MS analysis of conventionally propagated leaf and rhizome oil (CPAgLO and CPAgRO) and callus-induced leaf and rhizome oil (CIAgLO and CIAgRO) revealed a complex profile of 45 volatile compounds, predominantly monoterpenes, sesquiterpenes, and phenylpropanoids, confirmed by retention indices and mass spectral data. The analysis showed that in vitro callus-derived oils largely retained the same major constituents as conventionally propagated oils, indicating conservation of EO biosynthesis under culture conditions.

Monoterpenes such as α-pinene, camphene, β-pinene, myrcene, limonene, and eucalyptol were the predominant compounds in both conventionally propagated and callus-induced oils. Eucalyptol was the major component, representing 29–36% in leaves and 22–27% in rhizomes, while other monoterpenes, including β-pinene and camphor, were also abundant but slightly reduced in callus-derived samples. Oxygenated monoterpenes, such as borneol, fenchyl acetate, and α-terpineol, were present in both sample types, with guaiol showing a notable increase in callus-induced oils (7.13–7.19%) compared to propagated rhizome oil, suggesting a possible culture-mediated enhancement of specific compounds (Table 3). Sesquiterpenes, including caryophyllene, α-bergamotene, trans-β-farnesene, curcumene, and δ-selinene, were detected in trace to moderate amounts across all samples, with minor variations between propagated and callus-derived oils (Table 3). Phenylpropanoids such as methyl cinnamate and methyl eugenol were also retained *in vitro*, although methyl eugenol showed slightly lower levels in callus-induced samples.

Overall, GC-MS profiling demonstrated that callus induction preserves the chemical profile of EOs, with most major constituents conserved and certain compounds, such as guaiol and fenchyl acetate, exhibiting enhanced accumulation in callus-derived leaf and rhizome oils. This highlights the potential of in vitro culture for producing bioactive compounds while maintaining the characteristic phytochemical composition.

Total phenolic and flavonoid content:

The TPC and TFC varied significantly between oils derived from conventionally propagated and callus-induced *A. galanga* tissues (Table 4). Callus-induced leaf oil recorded the highest values (TPC: 84.38 ± 1.03 mg GAE/g; TFC: 75.33 ± 1.17 mg QE/g), which were significantly greater than conventionally propagated leaf oil (TPC: 70.25 ± 1.13; TFC: 61.92 ± 1.12). Similarly, callus-induced rhizome oil showed higher TPC (39.83 ± 0.95) and TFC (44.33 ± 0.72) compared to conventionally propagated rhizome oil (TPC: 33.44 ± 1.35; TFC: 37.46 ± 1.05). These findings indicate that callus induction enhances phenolic and flavonoid accumulation in both leaf and rhizome oils of *A. galanga.*

Antioxidant activity

DPPH assay results revealed strong free radical scavenging activity in the tested samples, comparable to the standard antioxidant ascorbic acid. This assay is a quick and reliable method commonly used to assess the antioxidant potential of plant EOs and bioactive compounds [11]**.** The results indicated that the callus-induced EOs exhibited stronger DPPH scavenging activity compared to the conventionally propagated EOs. The percentage inhibition increased in a concentration-dependent manner for all tested samples (Figure 1). At lower concentrations (10–20 µg/ml), CI AgRO and CP AgRO showed higher scavenging activities compared to ascorbic acid and the other EOs. At 100 µg/ml, all samples achieved inhibition above 90%, indicating strong radical scavenging potential.

The IC_{50} values, representing the concentration required to inhibit 50% of DPPH radicals, CI AgRO exhibited the lowest IC_{50} value (14 µg/ml), followed by CP AgRO (15 µg/ml) and CI AgLO (16 µg/ml), which were all slightly more potent than ascorbic acid (18 µg/ml). CP AgLO showed comparatively weaker activity, with an IC_{50} of 20 µg/ml. Overall, the results indicate that both *A. galanga* leaf and rhizome oils possess strong antioxidant activities, with CI AgRO demonstrating the highest radical scavenging potential. These findings suggest that *A. galanga* oils, particularly CI AgRO, may serve as promising natural antioxidant agents.

Discussion

***In vitro* culture establishment by indirect regeneration:**

The results clearly demonstrate that both the concentration of auxin (2,4-D) and its interaction with the cytokinin TDZ are critical for embryogenic callus induction in *A. galanga*. The highest response (76.84%) was achieved with 2 mg L^{-1} 2,4-D in combination with 0.3 mg L^{-1} TDZ, whereas higher levels of either regulator reduced induction efficiency. This pattern indicates a synergistic interaction between 2,4-D and TDZ, where auxin promotes cell dedifferentiation and cytokinin enhances morphogenic competence. Conversely, supra-optimal concentrations of 2,4-D favored unorganized callus proliferation and loss of embryogenic potential [12][13][14]. Within *A. galanga*, earlier studies corroborate our findings. Rhizome explants cultured on MS medium with 1.5 mg L^{-1} 2,4-D produced compact embryogenic callus, while higher concentrations were less effective [15]. Likewise, Rao et al. [6] demonstrated indirect organogenesis in *A. galanga* using 2 mg L^{-1} 2,4-D in combination with BAP or NAA, highlighting that an appropriate auxin–cytokinin balance is crucial for achieving morphogenic responses.

Comparable findings have been reported in other members of the Zingiberaceae family.

In *Curcuma amada*, somatic embryogenesis was achieved when 2,4-D (9.0 µM) was combined with low concentrations of cytokinins, specifically BA (8.88 µM) and NAA (2.7 µM), while excess auxin suppressed embryogenic responses [16]. Similarly, in *Zingiber zerumbet*, Mehaboob et al. [17] reported suitable callus induction on MS medium with moderate levels of BA (1.5 mg/L) and 2,4-D (0.3 mg/L). These parallels support the view that balanced auxin–cytokinin interactions are central to embryogenesis in Zingiberaceae.

Taken together, the present study confirms that intermediate concentrations of 2,4-D, particularly in combination with low levels of TDZ, are optimal for embryogenic callus induction in *A. galanga*. Such optimized protocols not only provide an efficient platform for large-scale plant regeneration but also open avenues for *in vitro* production of bioactive metabolites such as eucalyptol, a major phytoconstituent of this species.

Influence of growth regulators on shoot regeneration in *A. galanga*

The present study demonstrates that the choice and concentration of plant growth regulators (PGRs) play a pivotal role in regulating shoot regeneration from embryogenic cell suspensions of *Alpinia galanga*. Among the treatments tested, MS medium supplemented with 2 mg L^{-1} BA yielded the maximum regeneration frequency (75.24 ± 0.72%) and the highest number of shoots per callus (11.46 ± 0.12). However, an increase in BA concentration to 3 mg L^{-1} or the addition of kinetin resulted in a marked reduction in regeneration efficiency, suggesting that supra-optimal cytokinin levels exert inhibitory effects on morphogenesis in *A. galanga*.

These results are consistent with earlier reports on tissue culture of *A. galanga* and related Zingiberaceae members, where benzyladenine (BA/BAP) was identified as the most effective cytokinin for shoot induction. Rao et al. [6] noted that BA-supplemented callus cultures readily differentiated into shoots, underscoring its importance in inducing morphogenetic responses. Similarly, Borthakur et al. [5] and Pooja [4] reported that BA alone or in combination with low auxin concentrations promoted multiple shoot formation in *A. galanga*, reinforcing the strong cytokinin-dependence of this species. Additionally, Shamsudheen et al. [15] demonstrated that 1.0 mg L^{-1} BA was most effective for shoot regeneration from rhizome bud callus, with higher or combined cytokinin concentrations resulting in lower responses. In long-term culture experiments, Sahoo et al. [7] also highlighted BA as an essential component for sustained micropropagation, with kinetin and auxin combinations only supplementing, but not surpassing, BA-driven morphogenesis. Collectively, these findings corroborate that *A. galanga* exhibits a strong cytokinin specificity, with BA being the most consistent regulator of shoot regeneration, while elevated levels or supplementation with kinetin provide no significant advantage and may even reduce regeneration efficiency.

GC-MS analysis

The GC-MS profile obtained for conventionally propagated leaf and rhizome oils and callus-induced leaf and rhizome oils is broadly consistent with published compositions of *A. galanga* EOs: 1,8-cineole (eucalyptol) and camphor are repeatedly reported as dominant constituents of rhizome and leaf oils, while methyl cinnamate/methyl eugenol and several sesquiterpenes occur at lower but notable levels. For instance, Jirovetz et al. [2] reported high proportions of 1,8-cineole together with camphor, methyl cinnamate and guaiol in *A. galanga* rhizome and leaf oils, supporting our identification of eucalyptol as the major constituent.

Quantitatively, the higher abundance of eucalyptol in leaves compared to rhizomes, together with its substantial retention in callus-derived oils, demonstrates that *in vitro* cultures successfully preserve the characteristic phytochemical profile of *A. galanga*. Such retention of key constituents has also been successfully observed in several other members of the Zingiberaceae family [7][18][19]. Regional, seasonal, and methodological differences (plant provenance, developmental stage, plant parts, extraction technique, and GC conditions) commonly explain reported variation in absolute percentages of particular constituents across different studies [2][20][21][22][23]. The retention of most major constituents in callus oils despite some shifts in relative abundance is noteworthy and supports the idea that core monoterpene and phenylpropanoid biosynthetic pathways remain active in *in vitro* callus of *A. galanga*. The conservation of the major bioactive aroma compounds in callus-derived oils implies that *in vitro* production could be a viable alternative source for these constituents useful for standardized bioactivity assays or formulation, while the culture-mediated shifts in minor constituents (e.g., guaiol enrichment) may offer opportunities to selectively enhance desirable compounds via culture media optimization. Overall, these findings highlight the potential of *in vitro* culture for producing *A. galanga* EOs with both qualitative fidelity and opportunities for targeted metabolic modulation.

Total phenolic and flavonoid content

The enhanced levels of phenolic and flavonoid compounds observed in callus-induced oils of *A. galanga* suggest that *in vitro* cultures retain, and in some cases amplify, the biosynthetic potential of secondary metabolites. Phenolics and flavonoids are well-recognized contributors to antioxidant and pharmacological properties in Zingiberaceae species, and higher accumulation in callus-derived oils is consistent with reports from *in vitro* cultures of related taxa such as *Zingiber officinale* and *Kaempferia galanga*, where tissue culture systems produced elevated levels of polyphenolics relative to conventionally grown plants [7][23][24]. This enhancement may be attributed to stress responses associated with *in vitro* conditions, which are known to activate key biosynthetic pathways, particularly the phenylpropanoid pathway. The elevated TPC and TFC in callus-induced leaf oils compared to rhizome oils also aligns with earlier studies showing that leaves often accumulate higher levels of phenolics and flavonoids than underground tissues due to their direct exposure to light and metabolic activity [25][26][27][24]. Importantly, the successful retention of these metabolites in callus cultures reinforces the potential of tissue culture-derived oils as an alternative and sustainable source of bioactive compounds, with added opportunities for optimizing culture conditions to further enhance metabolite yields.

Antioxidant activity

The present study demonstrates that *A. galanga* EOs possess free radical scavenging activity, as evidenced by the DPPH assay. The antioxidant potential of the tested samples was concentration dependent, with all oils showing more than 90% inhibition at higher concentrations (100 µg/ml). Importantly, the callus-induced rhizome oil (CI AgRO) exhibited the strongest activity with the lowest IC_{50} (14 µg/ml), surpassing even the standard ascorbic acid (18 µg/ml).

This enhanced performance of CI AgRO, along with CP AgRO and CI AgLO, suggests that *A. galanga* oils are rich in bioactive compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. The observation that callus-induced oils displayed greater antioxidant activity than conventionally propagated oils highlights the potential of *in vitro* propagation methods as a sustainable alternative for producing bioactive compounds. Such approaches not only ensure a consistent yield of phytochemicals but also reduce dependence on natural populations of *A. galanga*, which is considered endangered due to overharvesting and habitat degradation [28][29]. These findings align with previous studies on Zingiberaceae plants and further demonstrate that *in vitro*-derived oils possess stronger antioxidant capacity compared to conventionally propagated counterparts [7][23][24]. Overall, the results show that both *A. galanga* leaf and rhizome EOs possess strong antioxidant activity, with CI AgRO exhibiting the highest potential. Given the endangered status of *A. galanga*, *in vitro* propagation and alternative cultivation methods offer sustainable options to conserve this species while providing a reliable source of its antioxidant compounds for pharmaceutical and nutraceutical applications.

Conclusion

The present study demonstrates that *A. galanga* can be efficiently regenerated *in vitro* through callus induction, with 2 mg L^{-1} 2,4-D combined with 0.3 mg L^{-1} TDZ identified as the most effective condition for embryogenic callus formation. BA at 2 mg L^{-1} proved optimal for shoot regeneration from embryogenic suspensions. GC–MS profiling confirmed that callus-derived oils largely retained the major constituents of conventionally propagated oils, with enhanced accumulation of certain compounds such as guaiol and fenchyl acetate. Moreover, callus-induced oils exhibited higher phenolic and flavonoid contents, which correlated with stronger antioxidant activity as evidenced by lower IC_{50} values in DPPH assays, particularly for CI AgRO. In summary, the findings highlight the potential of *A. galanga* EOs as natural antioxidant agents. Importantly, *in vitro* culture offers a sustainable and efficient alternative to conventional propagation, ensuring the production of valuable bioactive compounds while contributing to the conservation of this endangered species.

Acknowledgement

The authors express their gratitude to the president of SOA University, Bhubaneswar, Odisha, India, for providing the necessary instrumental amenities and valuable support.

Conflict of interest

The authors declare that there are no conflicts of interest.

Table 1: Effect of different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and thidiazuron (TDZ) on somatic embryogenic callus induction and proliferation in Murashige and Skoog medium

MS media with different phytohormones (mgL^{-1})	Rate of embryogenic callus induction
2,4-D(1)	39.78
2,4-D(2)	48.63
2,4-D(3)	20.65
2,4-D(2) + TDZ(0.2)	67.52
2,4-D(2) + TDZ(0.3)	76.84
2,4-D(2) + TDZ(0.5)	49.57
2,4-D(3) + TDZ(0.2)	37.38
2,4-D(3) + TDZ(0.5)	49.95

Values represent the mean rate of embryogenic callus induction calculated from 15 plants analyzed per treatment.

Table 2: Effect of different growth regulators on plantlet regeneration from embryogenic cell suspension culture of Alpinia galanga

Growth regulators (mg L^{-1})	Regeneration percentage (%)	Number of shoots per callus inoculated
BA(2)	75.24 ± 0.72	11.46 ± 0.12
BA(3)	58.63 ± 0.55	8.62 ± 0.33
BA(2) + Kn(0.5)	50.67 ± 0.34	6.29 ± 0.22
BA(2) + Kn (1.0)	37.28 ± 0.25	4.83 ± 0.34

Table 3: GC-MS Analysis of normal and cultured sample

Sl no.	Compound	RI[a]	RI[b]	CPAgLO	CIAgLO	CPAgRO	CIAgRO	ID[c]
1	α-pinene	931	932	*	*	6.79	6.81	RI, MS
2	camphene	947	946	*	*	9.28	9.31	RI, MS
3	Sabinene	970	969	*	*	0.16	0.2	RI, MS
4	β-pinene	976	974	10.89	11.01	9.68	10.61	RI, MS
5	Myrecene	986	988	*	*	0.81	0.88	RI, MS
6	P-cymene	1021	1020	*	*	*	*	RI, MS
7	O-cymene	1022	1022	*	*	0.81	0.89	RI, MS
8	Limonene	1026	1024	*	*	5.1	5.4	RI, MS
9	Eucalyptol	1030	1026	**29.61**	**35.53**	**21.61**	**27.21**	RI, MS
10	β-ocimene	1043	1044	*	*	*	*	RI, MS
11	Y-terpinene	1055	1054	*	*	0.28	0.29	RI, MS
12	Fenchone	1087	1083	*	*	0.31	0.31	RI, MS
13	Terpinolene	1089	1086	0.36	0.43	0.16	0.16	RI, MS
14	Fenchol exo	1118	1118	*	*	0.46	0.5	RI, MS
15	Camphor	1146	1141	9.96	10.01	2.31	2.32	RI, MS
16	Borneol	1170	1165	3.31	3.44	1.08	1.21	RI, MS
17	4-Terpineol	1175	1174	0.69	0.99	*	*	RI, MS
18	α-terpineol	1179	1186	1.26	1.27	*	*	RI, MS
19	Neo-dihydrocarveol	1197	1193	*	*	3.67	3.98	RI, MS
20	Myrtenal	1193	1195	0.94	1.02	*	*	RI, MS
21	Fenchyl acetate	1218	1218	*	*	15.54	15.55	RI, MS
22	Bornylacetate	1282	1284	1.32	1.35	*	*	RI, MS
23	α-terpineol acetate	1343	1346	*	*	0.45	0.45	RI, MS
24	α-copaene	1377	1374	*	*	1.73	1.79	RI, MS
25	Methyl cinnamate	1381	1376	1.28	1.33	1.27	1.29	RI, MS
26	Methyl eugenol	1400	1403	8.99	9.34	*	*	RI, MS
27	Caryophyllene	1400	1408	1.02	1.11	*	*	RI, MS
28	β-patchoulene	1374	1379	*	*	*	*	RI, MS
29	α-gurjunene	1408	1409	*	*	*	*	RI, MS
30	β- gurjunene	1429	1431	0.98	1.06	*	*	RI, MS
31	α-Bergamotene	1435	1432	1.04	1.21	*	*	RI, MS
32	trans-β-Farnesene	1438	1440	1.9	2.36	*	*	RI, MS

33	Curcumene	1475	1479	1.34	1.41	*	*	RI, MS
34	Germacrene D	1485	1484	*	*	0.21	0.25	RI, MS
35	δ- Selinene	1496	1492	1.98	2.1	*	*	RI, MS
36	β-himachalene	1495	1500	*	*	0.16	0.18	RI, MS
37	β-Bisabolene	1502	1505	1.23	1.31	*	*	RI, MS
38	α-Farnesene	1505	1505	0.50	0.51	*	*	RI, MS
39	Germacrene A	1510	1508	*	*	*	*	RI, MS
40	β-Sesquiphellandrene	1516	1521	0.95	1.31	*	*	RI, MS
41	Caryophyllene oxide	1587	1582	1.09	1.11	*	*	RI, MS
42	Carotol	1597	1594	*	*	*	*	RI, MS
43	Guaiol	1608	1600	1.69	1.70	7.13	7.19	RI, MS
44	α-cadinol	1668	1652	*	*	*	*	RI, MS
45	Selin-11-en-4α-ol	1665	1658	*	*	3.15	3.18	RI, MS

**Compounds not detected. RI[a]: Retention indices on Elite-5 column, experimentally determined using homologous series of C8-C20 n-alkanes. RI[b]: Retention index taken fr literature. ID[c] : Identification methods: MS, comparison of mass spectra with NIST library, Adams, 2007; RI, comparison of retention index with those reported in literature.*

Table 4: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of both Conventionally Propagated and Callus induced Alpinia galanga

Sl.No	Plant Parts extract	TPC	TFC
1	Conventionally propagated Ag leaf oil	70.25±1.13[a]	61.92±1.12 [a]
2	Callus induced Ag leaf oil	84.38±1.03[b]	75.33±1.17[b]
3	Conventionally propagated Ag rhizome oil	33.44±1.35[a]	37.46±1.05 [a]
4	Callus induced Ag rhizome oil	39.83±0.95[a]	44.33±0.72[b]

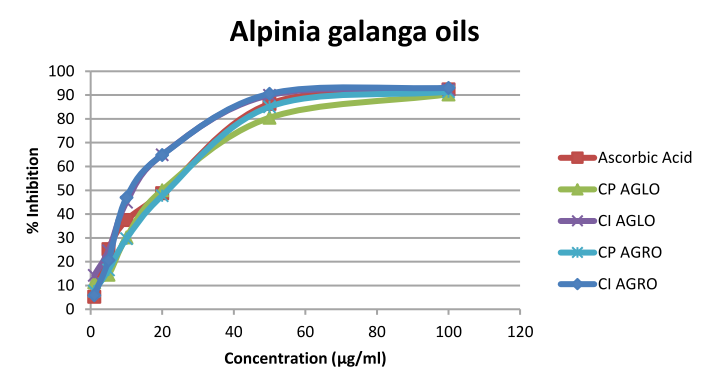

Fig. 1: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of essential oils of both Conventionally Propagated and Callus induced Alpinia galanga

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