doi.org/10.36721/PJPS.2024.37.3.REG.583-590.1

Quantification and comparison of major secondary metabolites and antioxidant efficacy in *Gardenia jasminoides* mother plant, callus and suspension culture: an exploratory study to enhance bioactive compounds

Durga Anusha Sampath Kumar[1] and Deepa Sankar Parasurama[2]*

[1]School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore, Tamil Nadu, India.
[2]School of Agricultural Innovations and Advanced Learning, Vellore Institute of Technology, Vellore, Tamil Nadu, India.

Abstract: *Gardenia jasminoides* suspension culture has gained recognition as a functional approach for bioactive component development in the pharmaceutical industries but exhibits limited biomass accumulation and secondary metabolite production. This study presents the first record of maximum biomass production and demonstrates the cumulative levels of phenols, flavonoids and terpenoids observed through the growth trajectory of *G. jasminoides* suspension culture. Successful callus induction was obtained from leaf explants cultured on Murashige and Skoog (MS) medium augmented with a standardized conjunction of 1 mg/L of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.5 mg/L kinetin (KT). The experimental outcomes revealed that on the 35th day, the *in vitro* suspension culture exhibited the highest biomass accumulation which was 5.43 times greater than the initial inoculation level. The study quantified total phenols, flavonoids, and terpenoids present in leaf explants, callus cultures, and suspension cultures and determined antioxidant efficacy. Findings suggest that an optimized growth regulator in *G. jasminoides* suspension culture significantly increases biomass accumulation. Quantification of secondary metabolites offers a promising path for future enhancement of their yield through elicitation and holds the potential to achieve extensive yield of cost-effective bioactive components.

Keywords: *Gardenia jasminoides*; friable calli; suspension culture; secondary metabolites; antioxidant efficacy

INTRODUCTION

Gardenia jasminoides is a highly sought-after perennial aromatic shrub that falls under the taxonomic classification of the *Rubiaceae* family. This plant species is characterized by a delightful scent and white flowers. Initially, native to China and Japan, *G. jasminoides* plants have gradually expanded their distribution and are now found in various regions across the globe (Chen *et al.*, 2020). *G. jasminoides* is widely recognized for its aesthetic appeal and therapeutic properties, owing to the rich content of secondary metabolites possessing significant antioxidants and pharmaceuticals, aiding in combating pathogenic microorganisms (Reddy *et al.*, 2021). Besides, *G. jasminoides* crude extract is used as an anti-angiogenic agent and as a treatment for diabetes, haemorrhage, cardiovascular diseases, nerve disorders, dental pain, burns, wounds, muscle injuries, skin irritation, jaundice, hepatitis, and pancreatitis (Ran *et al.*, 2021; Hussain *et al.*, 2019; Manickam *et al.*, 2014).

Plant tissue culture is an emerging technique that involves the cultivation of plant cells under extreme aseptic conditions, necessitating the application of a nutritive medium for the growth and enhancement of components that can encourage the proliferation and differentiation of plant tissues and cells in a controlled manner. In the realm of modern plant science, the utilization of *in vitro* culture techniques has revolutionized various aspects, offering unprecedented opportunities for the production of plants that are free from diseases, enabling the cultivation of healthy specimens, increasing the yield of economically viable plant metabolites and facilitating the rapid multiplication of extinct plant genotypes. Recent advancements in plant tissue culture have shed light on the significance of callus cultures and suspension cultures as pivotal techniques for the replication and activation of metabolic pathways in plant cultures (Salim and Habeeb, 2018; Chandran *et al.*, 2020). Compared to organ and tissue culture techniques, *in vitro* cell suspension culture techniques provide an efficient means of achieving industrial-level secondary metabolite synthesis, rendering them a preferred choice for producing naturally occurring bioactive chemicals on a commercial scale (Fazili *et al.*, 2022). The application of plant suspension cultures has witnessed a notable surge in importance across diverse domains, including the formation of naturally developed nutritional additives and bioactive components. Moreover, the advantages of *in vitro* cell suspension culture remain consistent, irrespective of seasonal variations, ensuring the production of pathogen-free bioactive compounds while also stimulating diverse genes, thereby fostering the emergence of novel secondary metabolites with distinctive characteristics (Abdulhafiz *et al.*, 2022). Further, it offers distinctive benefits in terms of the

**Corresponding author:* e-mail: pdeepasankar@vit.ac.in

enhanced ease of purification of the resulting bioactive compounds compared to field-grown plants, exhibiting a higher level of consistency relative to the quality, thereby facilitating compliance with standards of good manufacturing practices (Arya *et al.,* 2020; Bapat *et al.,* 2023). Besides the favorably high productivity and enhanced product quality obtained through *in vitro* suspension culture, a wide range of intricate bioactive compounds spanning agrochemicals, pharmaceuticals, fragrances, and flavors can be successfully synthesized. (Dastmalchi *et al.,* 2019). Equally, the bioactive compounds present in *G. jasminoides* are of paramount importance in both the pharmaceutical and cosmetic sectors because of their distinctive attributes. This study elucidates the yield of secondary metabolites released during the suspension process.

To encourage the implementation of *G. jasminoides in vitro* suspension culture in industrial settings, it is imperative to acquire a communal comprehension of the existence of bioactive compounds and the temporal dynamics of biomass growth. Therefore, the application of batch suspension culture in *in vitro* analysis fosters an extensive understanding of cell proliferation patterns exhibited by *G. jasminoides* suspension cultures via cultivating a predetermined quantity of cells within a standardized volume of culture medium subjected to varying combinations of growth regulators in a favorable environment (Corbin *et al.,* 2020; Ahmad *et al.,* 2020). In the context of suspension culture, it is worth noting that plant cells typically aggregate, making the measurement of optical density (OD) unsuitable for assessing their growth. Consequently, in this particular investigation, we evaluated the growth of the *G. jasminoides* suspension culture employing the settled cell volume (SCV) technique as a metric that enabled us to estimate and monitor the growth of the culture in an effective way (Karimzadeh *et al.,* 2019; Siddiqui *et al.,* 2023). Limited information regarding callus induction in *G. jasminoides* is currently available. Liu *et al.,* 2018 and Krasteva, 2022 have recently published findings on the adoption of *G. jasminoides* suspension cultures for optimizing growth media and synthesizing secondary metabolites. To date, there is a dearth of literature addressing specific inquiries into biomass accumulation and overall secondary metabolite content within *G. jasminoides* suspension cultures. Although data comprehensively documenting total phenol, total flavonoid and total terpenoid levels in *in vitro* suspension cultures are yet to be compiled, prior research has reported on total phenol and total flavonoid content in *G. jasminoides* callus cultures without delving into the total terpenoid content. The absence of quantification of phenol, flavonoid, and terpenoid content in published suspension culture research necessitates the present investigation into the assessment of major secondary metabolites and antioxidant efficacy in *G. jasminoides* suspension cultures. Given the potential utilization of suspension cultures across diverse commercial and pharmaceutical domains, this study endeavors to explore (i) the growth kinetics of *G. jasminoides* suspension cultures by discerning conjunctions of plant growth regulators; (ii) the quantification of total phenol, flavonoid and terpenoid content; and (iii) the evaluation of antioxidant efficacy within leaf explants, calli and suspension cultures.

MATERIALS AND METHODS

Plant collection and maintenance

Plants were collected from Coimbatore, Tamil Nadu, India and maintained in a light-shaded environment at VIT Greenhouse (fig. 1a), Vellore, Tamil Nadu, India. The plants used in this study were authenticated by VIT Herbaria and given a voucher number, VITOD01-208.

Callus induction

Murashige and Skoog (MS) medium augmented with plant growth regulators 2,4-Dichlorophenoxyacetic acid (2,4-D) (varying from 1 mg/L to 4 mg/L), Naphthalene acetic acid (NAA) (3 mg/L) and kinetin (KT) (0.5 mg/L) was utilized for callus induction of *G. jasminoides*. The media's pH was modified to 5.8 and 0.7% agar was used for solidification. The media were sterilized for 20 min at 121 ºC. The leaves from 4-week-old plants were segmented by approximately 1 cm and disinfected with Tween-20 and 0.1% mercuric chloride for 5 min, followed by 2-3 washes before treatment with 70% ethanol for a few seconds (Chakraborty *et al.,* 2013; Din *et al.,* 2022). Leaf explants were inoculated on MS media and cultured at 25±2 ºC under darkness. Cultures of the best-responded combination were sub-cultured once every four weeks to obtain friable calli for *in vitro* suspension culture preparation.

Suspension culture and growth kinetics

The optimal response combination devoid of agar with a pH of 5.8 was utilized for further culture studies. Friable calli obtained from the sixth subsequent subculture were employed to initiate the suspension cultures. Approximately 1 g of actively multiplying (during the 20th day) friable calli was suspended with 80 ml liquid MS media, cultivated in a gyratory shaker (120 rpm) and cultured at 25±2 ºC under darkness. The cell growth curve was established by employing the SCV method to estimate growth kinetics once in 5 days for 6 weeks (Farjaminezhad *et al.,* 2013; Siddiqui *et al.,* 2023).

Preparation of sample extraction

2 g of fresh samples (leaf explant, callus, and *in vitro* suspension culture) were extracted with 10 ml of 80% methanolic solvent and kept overnight in a shaker at 120 rpm at ambient temperature. The extract was sonicated for 15-20 min and 5 min centrifugation at 6000 rpm. The supernatant fluid was retained and maintained at -20ºC (Gabr *et al.,* 2017; El-Ashry *et al.,* 2019).

Table 1: Impact of various plant growth regulator combinations on callus occurrence time, callus response rate and morphological characters

Treatment	Plant growth regulators (mg/L)			Callus induction (days)	Callus response %	Callus nature and color
	2,4-D	NAA	KT			
1	1	0	0.5	16.6±0.44^{b}	100^{a}	Semi-friable, pale yellow to pale white
2	2	0	0.5	19±0.69^{a}	93±3.33^{ab}	Semi-friable, pale white
3	3	0	0.5	20±0.75^{a}	87±3.33^{ab}	Semi-friable, pale yellow to pale white
4	4	0	0.5	20±0.91^{a}	67±6.66^{c}	Semi-friable, pale yellow to pale white
5	3	0.5	0.5	20±0.74^{a}	80±5.77^{bc}	Semi-friable, pale yellow
6	3	1	0.5	19±0.81^{a}	93±6.66^{ab}	Semi-friable, pale yellow to pale white

For each treatment, 30 explants were maintained and each experiment was carried out in triplicates. Callus induction in days and callus response % are shown as the mean value ± SE. Values not connected by the identical superscript alphabet are critically distinct by Duncan's multiple range test at a 5% probability of occurrence. SE- Standard Error.

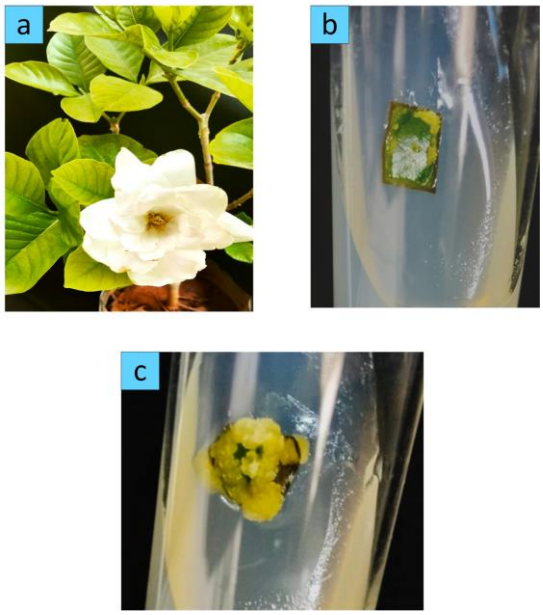

Fig. 1: a *G. jasminoides* plant maintained at VIT Greenhouse under light shade; b callus induction on the margin of the leaf explant; c aggregated semi-friable calli over the course of time from callus induction

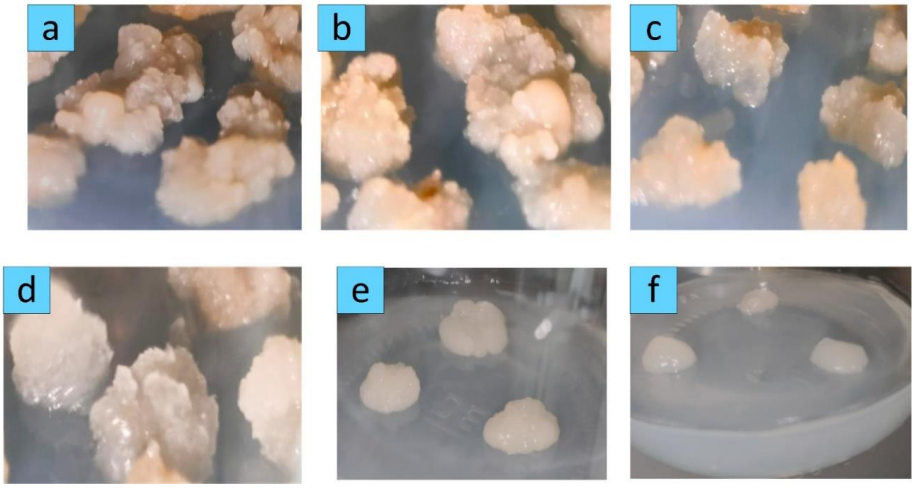

Fig. 2: a & b First and second subcultures of semi-friable calli from 2,4-D (1 mg/L) and KT (0.5 mg/L) with 0.7% agar on MS media; c & d third and fourth subcultures of semi-friable calli from 2,4-D (1 mg/L) and KT (0.5 mg/L) with 0.65% agar on MS medium; e & f fifth and sixth subcultures of complete friable and translucent callus from 2,4-D (1 mg/L) and KT (0.5 mg/L) with 0.6% agar on MS medium

Quantification of total content of phenol

The Folin-Ciocalteu (FC) procedure was utilized to quantify the total content of phenol employing gallic acid as standard and represented as gallic acid equivalents (GAE). 1 ml of each sample extract was diluted with 10% FC reagent (5 ml) before 2% sodium carbonate (4 ml) was added and incubated at ambient temperature for 1h and at 765 nm, the test absorbance was assessed. As a control, methanol was utilized (Truong *et al.*, 2019).

Quantification of total content of flavonoid

The aluminium chloride technique was utilized to estimate the total content of flavonoids using quercetin as standard and represented as quercetin equivalents (QE). The test mixture was established with extract (0.5 ml), deionized water (0.5 ml), and 5% sodium nitrite solution (0.3 ml) before incubation at 25 ºC for 5 min. The mixture was then immediately treated with 10% aluminium chloride (0.3 ml) and 1 M sodium hydroxide (2 ml) and at 510 nm, the absorbance value was evaluated (Sankhalkar and Vernekar, 2016).

Quantification of total content of terpenoid

The protocol devised by Lukowski *et al.*, (2022) was utilized to estimate the total terpenoid content using linalool as standard and represented as linalool equivalents (LE). 200 µl sample extract was added to chloroform (1.5 ml), vortexed thoroughly and remained for 3-4 min. Following, 100 µl sulphuric acid was introduced and incubated for 2 h (<5 min for standard solution) under darkness at ambient temperature. A reddish-brown precipitate formed and the supernatant fluid was decanted and vortexed using 95% methanol (1.5 ml). The absorbance was assessed at 538 nm. As a control, 95% methanol was used.

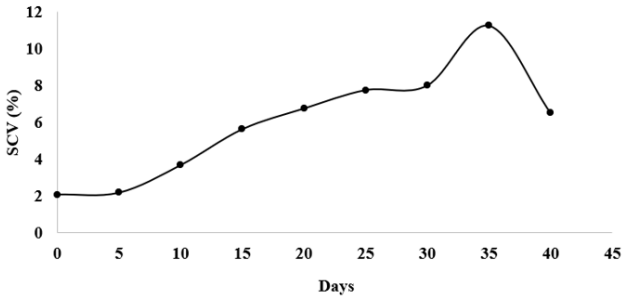

Fig. 3: Growth kinetics of *G. jasminoides in vitro* suspension culture via 2,4-D (1 mg/L) and KT (0.5 mg/L) on liquid MS medium.

Evaluation of antioxidant efficacy through DPPH radical inhibition test

The protocol devised by Hendel *et al.*, (2021) was utilized to evaluate antioxidant efficacy with minor modifications. 1 ml of each sample extract at distinct levels (25, 50, 75, and 100 µl/ml) was diluted with 0.1 mM DPPH (2 ml) and vortexed and left for 30-45 min under darkness at ambient temperature. At 517 nm, the test absorbance was assessed. The inhibition percentage was evaluated utilizing the formula: DPPH radical inhibition (%) = $(A_C-A_S/A_C) \times 100$, where A_C denotes control absorbance and A_S denotes sample absorbance.

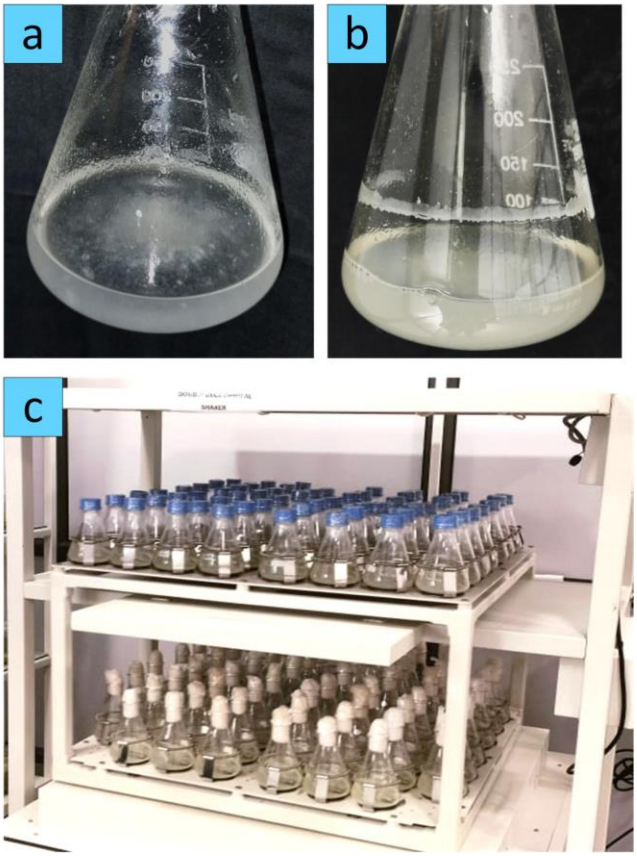

Fig. 4: a Suspension culture of *G. jasminoides* from 2,4-D (1 mg/L) and KT (0.5 mg/L) on MS liquid media on the day of inoculation; b biomass accumulation of *G. jasminoides* suspension culture at day 35; c cell suspension cultures of *G. jasminoides* on a double deck gyratory shaker

STATISTICAL ANALYSIS

Triplicates of each experiment were executed and represented as mean ± SE (Standard Error). The outcomes were examined by One-way Analysis of variance. Duncan's multiple range test was employed to analyze means using IBM SPSS software (version 27), with a 5% probability of occurrence.

RESULTS

Callus induction

The present study examined how plant growth regulators affected the callus induction of *G. jasminoides* at various concentrations, as well as the ratios between these growth regulators and callus response percentages (table 1). Typically, between the 16th and 20th days, a pin head

Table 2: Total phenol, flavonoid and terpenoid levels of different culture systems of *G. jasminoides*

Source	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg LE/g)
Leaf explant	34.58±0.29a	182.46±0.88a	265.55±11.46a
Callus	3.12±0.1de	2.47±0.4de	86±3.49b
SC0	4.18±0.36cd	2.99±0.15de	58.96±5.64cd
SC5	4.41±0.42bc	4.48±0.38cd	61.3±2.05cd
SC10	3.43±0.08cde	6.98±0.15c	87.96±2.04b
SC15	3.29±0.27cde	6.5±2.33c	61.7±7.47cd
SC20	2.73±0.04e	5.1±0.94cd	66.4±8.22c
SC25	3.09±0.17de	3.34±1.01de	52.63±2.95cd
SC30	3.23±0.42de	4.4±0.63cd	57.73±4.52cd
SC35	5.32±0.8b	16.94±0.4b	47.16±2.55d
SC40	2.83±0.1e	0.97±0.08e	46±1.05d

The experiment was replicated thrice. Duncan's Multiple Range Test (P≤0.05) was accomplished utilizing IBM SPSS Statistics (27.0). Treatment means for a variable have been indicated with an identical letter if they did not critically differ from its consecutive treatment in descending order. SC-Suspension culture.

Table 3: DPPH free radical inhibiting efficacy of distinct cultures of *G. jasminoides* at different concentrations

Extract concentration (µl/ml)	Antioxidant activity (Inhibition %)		
	Leaf explant	Callus	Suspension culture
25	18.6±2.12d	0.82±0.05j	3.32±0.14gh
50	32.76±2.56c	1.92±0.05i	7.88±0.09fg
75	48.57±2.79b	3.11±0.10gh	11.03±0.35ef
100	64.27±4.71a	4.21±0.05gh	15.76±0.19de

The experiment was replicated thrice. Duncan's Multiple Range Test (P≤0.05) was accomplished utilizing IBM SPSS Statistics (27.0). Treatment means for a variable have been indicated with an identical letter if they did not critically differ from its consecutive treatment in descending order.

callus initiated to form on the margin of the leaf explants (fig. 1b) and then periodically spread outward until it eventually covered the entire explant (fig. 1c). Since the cultures were incubated under dark conditions, semi-friable calli that ranged in color from bright white to pale white were developed and their nature and color were almost identical in all treatments.

Establishment of friable calli

Treatment 1 (1 mg/L 2,4-D and 0.5 mg/L KT) had a significant response and the shortest callus occurrence time and was hence chosen for further *in vitro* investigations. The densely packed calli were cultivated in fresh MS medium augmented with the optimal PGR combination with 0.7% agar and adjusted to 5.8 pH. Subsequent subcultures were performed once in four weeks with a reduction in the agar concentration. For instance, firstly, the media was administered with 0.7% agar in subcultures 1 and 2 (fig. 2 a, b), then with 6.5% agar in subcultures 3 and 4 (fig. 2 c, d), and eventually with 6% agar in subcultures 5 and 6 (fig. 2 e, f).

Growth kinetics of G. jasminoides cell suspension culture

Friable and translucent callus clusters developed through the sixth subculture augmented with 2,4-D (1 mg/L) and KT (0.5 mg/L) were employed to commence *in vitro* suspension cultures. Eventually, the growth kinetics of suspension cultures of *G. jasminoides* were ascertained by examining the cell growth in liquid MS media with similar PGR concentrations. As an outcome, the SCV (%) of the suspension cultures was determined once in five days to measure cell growth. A sigmoidal cell growth cycle was observed with four distinct phases: lag, log, or exponential, standard or stationary, and death or decline. The duration of a complete growth cycle of *G. jasminoides* suspension cultures was observed to be 40 days (fig. 3).

Determination of total content of phenol, flavonoid and terpenoid

Variations in total phenols, flavonoids, and terpenoids levels were observed in *G. jasminoides* mother plant, calli, and suspension cultures at five-day intervals, till the decline phase (table 2). The mid-log phase of *G. jasminoides* cell growth exhibited the lowest secondary metabolite content, coinciding with a period of active cell growth and generation of critical biomass. The experimental outcome showed the maximum levels of phenol and flavonoid compounds on the 35th day, exhibiting a notable hike of 1.27-fold and 5.67-fold respectively from the day of inoculation. The terpenoid content reached its peak level on the 10th day, representing a substantial rise of 1.49-fold compared to the initial value measured on the day of inoculation. Following the 10th day, the total terpenoid content gradually decreased.

Evaluation of antioxidant efficacy through DPPH radical inhibition test

The technique employed to inhibit DPPH free radicals was utilized to assess the chain-breaking capacity during

the active multiplication stage of protein and oxidation of lipids. The outcomes indicate that all the methanolic extract samples exhibited an increase in DPPH scavenging activity that was dependent on the dosage administered (table 3). The antioxidant activity of leaf explant extracts exhibited higher radical scavenging efficacy, suggesting a substantial degree of antioxidant potential whereas callus established the lowest radical inhibition percentage as expected. The *in vitro* suspension culture (highest biomass accumulation on the 35th of the growth cycle), exhibited a better level of antioxidant activity when compared to the callus culture.

DISCUSSION

A prior study revealed that to a significant level, the types, number, concentration, and ratio of plant growth regulators exhibited a substantial influence on the induction of callus (Ahmadpoor *et al.*, 2022; Liu *et al.*, 2018). The calli that occurred in all treated groups had similar morphological characteristics during each course of treatment. However, there were indispensable differences in the callus induction day and callus response rate between treatments. Among the five other treatments, the first treatment resulted in the shortest callus induction time with a 100% response rate. Following the first treatment, the growth regulator combination of the second and sixth treatments demonstrated comparable callus occurrence time with improved callus response percentages. Moreover, the optimal NAA concentration from prior studies and the 2,4-D and KT combinations were examined in treatment 5 and treatment 6 as three growth regulator combinations. However, these three combinations also offered a callus initiation time that was comparable to that of the other two combinations. Although KT concentration was unchanged in all treatments and the ideal concentration of NAA was used, 2,4-D served a crucial function in *G. jasminoides* callus induction and response rate. For instance, the lowest 2,4-D concentration in treatment 1 had the shortest callus induction time with a high response rate, in contrast to the highest 2,4-D concentration in treatment 4 which had an extended callus induction time with the lowest response rate percentage.

The reduction of agar concentration in the media enabled the development of friable calli for *in vitro* cell suspension culture studies. An extensively derived callus culture with an ideal appearance was achieved in the sixth subculture, which was adequate for the subsequent suspension culture experiments. Kong *et al.*, 2023 also stated that the emergence of the *in vitro* suspension culture was facilitated by several subcultures, which is consistent with our research findings. Farjaminezhad *et al.*, 2013 studied the rate of growth kinetics of *Papaver bracteatum in vitro* suspension cultures using SCV (%) augmented with ascorbic acid (15 mg/L), NAA (1 mg/L) and 6-Benzylaminopurine (1 mg/L), which demonstrated a lag phase lasting for 2 days, followed by a log phase spanning 14 days. Upon completion of the 14th day, the cells transitioned to the stationary phase. The cellular growth rate of *G. jasminoides* exhibited a slow pace pattern during the initial five-day period (lag phase) indicating that on inoculation a period of five days was required for the cells to acclimatize to the fresh culture conditions. As the experimental cultures progressed, a noticeable upward trend in cell growth was observed starting from the fifth day. The cells exhibited rapid growth, resulting in an apparent increase in SCV (%). Specifically, SCV (%) showed a remarkable 3.57-fold increase from the fifth day onward, spanning 20 days (exponential phase: 5th day to 25th day). The growth rate was stable from the 25th day, with the highest biomass accumulation observed on the 35th day. A prior study on *G. jasminoides* suspension culture revealed a 3.96-fold increase in biomass accumulation (Liu *et al.*, 2018). Notably, in this study, the peak biomass (fig. 3) reached 5.43 folds than the initial volume (fig. 3). Beyond the 35th day of the growth cycle, a sudden fall in biomass was observed, indicating the depletion of nutrients within the culture medium.

Field-grown leaf explants exhibited the highest levels of phenol, flavonoid and terpenoid content, followed by *in vitro* suspension cultures. Callus cultures recorded the lowest metabolite contents among the tested culture systems. Plants exhibit a remarkable ability to synthesize large amounts of critical secondary metabolites or bioactive components in an apparent reaction to various forms of biotic (herbivores, insects, nematodes, microorganisms, weeds) as well as abiotic stressors (temperature, salinity, UV-radiation, drought, floods, heavy metals). Callus is a cluster of undifferentiated cells that mainly participate in cellular division and develop under favorable growth conditions, resulting in reduced development of bioactive components (Efferth, 2019; Pan *et al.*, 2020). Likewise, *in vitro* cell suspension cultures, which are developed under controlled conditions with optimal temperatures, suitable environmental factors and regular nutrient supplies do not experience the same range of stresses as their counterparts in natural ecosystems (Umar *et al.*, 2021). The levels of bioactive components are known to be altered by cell adaptation to new environmental conditions (Aziz *et al.*, 2017; Li *et al.*, 2020). Mathew and Sankar (2014) reported that the application of an abiotic elicitor (methyl jasmonate) effectively aids in the synthesis of major secondary metabolites in *Ocimum* species. The current study determined that *G. jasminoides* cell suspension cultures contained approximately 1/6th, 1/10th and 1/3rd of the total phenol, flavonoid and terpenoid compounds respectively compared to leaf explants, giving scope for analyzing the impact of elicitors in upscaling the improved yield of bioactive components, thereby providing an alternative strategy to mass multiply valuable metabolites as raw materials for pharmaceutical and cosmetics-based industries.

The enhancement in scavenging activity in suspension culture could be accorded to the existence of bioactive components due to the minimal stress exerted on the cells cultured by agitating at 120 rpm. The observed antioxidant impacts have been hypothesized in the capability of compounds to donate molecules of hydrogen (Muthukrishnan *et al.,* 2018; Nurmazela *et al.,* 2022). The field-grown leaf explant extract exhibited superior antioxidant activity, which could be due to its abundant secondary metabolite accumulation. However, it is noteworthy that *in vitro* cultures have the potential to achieve a comparable quantity of bioactive components through elicitors that function as signals recognized by cell membrane receptors, thereby stimulating metabolic pathways and augmenting the development of bioactive components (Halder *et al.,* 2019; Jeyasri *et al.,* 2023).

CONCLUSION

The current study provides extensive information about the major secondary metabolites found in the *in vitro* suspension culture of *G. jasminoides*, providing insights into fluctuations in metabolite levels and antioxidant properties, and filling a notable void in the existing literature by elucidating the metabolite composition previously unexplored. Our study revealed that careful adjustment of plant growth hormones in a controlled setting with regular agitation enhances the development of bioactive components in the *in vitro* suspension cultures compared to callus cultures owing to its faster growth rates and homogeneity, which align with its growth cycle dynamics. Quantifying the total metabolite content in *G. jasminoides* suspension culture is of paramount significance owing to its holistic perspective, enabling broader investigations into the influence of diverse techniques on bioactive compound synthesis that are pivotal for advancing research in the pharmacological and beauty aids industries. Given the bounded number of published *in vitro* suspension studies on *G. jasminoides*, this research serves a vital function in unraveling the biosynthetic pathways of bioactive components by employing omics techniques and metabolite enhancement via elicitation, laying the groundwork for future analyses.

REFERENCES

Abdel-Aziz SM, Elsoud MMA and Anise AA (2017). Microbial biosynthesis: A repertory of vital natural products. In: *Food Biosynthesis*. Academic Press. pp. 25-54.

Abdulhafiz F, Mohammed A, Reduan MFH, Kari ZA, Wei LS and Goh KW (2022). Plant cell culture technologies: A promising alternatives to produce high-value secondary metabolites. *Arab. J. Chem.,* **15**(11): 104161.

Ahmad TA, Al-Mahdawe MM and Nadir DS (2020). Effect of methyl jasmonate on the production of furanocoumarins in cell suspension cultures of *Ruta graveolens* L. *PCTOC,* **143**: 565-571.

Ahmadpoor F, Zare N, Asghari R and Sheikhzadeh P (2022). Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production *in vitro* cultures *Melia azedarach* L. *AMB Express,* **12**(1): 1-12.

Arya SS, Rookes JE, Cahill DM and Lenka SK (2020). Next-generation metabolic engineering approaches towards development of plant cell suspension cultures as specialized metabolite producing biofactories. *Biotechnol. Adv.,* **45**: 107635.

Bapat VA, Kavi Kishor PB, Jalaja N, Jain SM and Penna S (2023). Plant cell cultures: Biofactories for the production of bioactive compounds. *Agronomy,* **13**(3): 858.

Chakraborty N, Banerjee D, Ghosh M, Pradhan P, Gupta NS, Acharya K and Banerjee M (2013). Influence of plant growth regulators on callus mediated regeneration and secondary metabolites synthesis in *Withania somnifera* (L.) Dunal. *PMBP,* **19**: 117-125.

Chandran H, Meena M, Barupal T and Sharma K (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotechnol. Rep.,* **26**: e00450.

Chen L, Li M, Yang Z, Tao W, Wang P, Tian X and Wang W (2020). *Gardenia jasminoides* Ellis: Ethno-pharmacology, phytochemistry and pharmacological and industrial applications of an important traditional Chinese medicine. *J. Ethnopharmacol.,* **257**: 112829.

Corbin JM, McNulty MJ, Macharoen K, McDonald KA and Nandi S (2020). Technoeconomic analysis of semi continuous bioreactor production of bio-pharmaceuticals in transgenic rice cell suspension cultures. *Biotechnol. Bioeng.,* **117**(10): 3053-3065.

Dastmalchi T, Omidi M, Azizinezhad R, Rezazadeh S and Etminan A (2019). Effects of methyl jasmonate and phloroglucinol on thebaine and sanguinarine production in cell suspension culture of Persian poppy (*Papaver bracteatum* Lindl.). *Cell. Mol. Biol.,* **65**(3): 11-17.

Deng X, Xiong Y, Li J, Yang D, Liu J, Sun H and Yang M (2020). The establishment of an efficient callus induction system for lotus (*Nelumbo nucifera*). *Plants*, **9**(11): 1436.

Din A, Qadri ZA, Wani MA, Iqbal S, Malik SA, Bhat ZA and Banday N (2022). Developing an efficient *in vitro* callusing and regeneration protocol in *Dendranthema*× *grandiflorum* Kitam. *JCSB,* **25**(4): 393-405.

Efferth T (2019). Biotechnology applications of plant callus cultures. *Engineering,* **5**(1): 50-59.

El-Ashry AAEL, Gabr AMM, Arafa NM and El-Bahr MK (2019). Rutin accumulation in gardenia calli cultures as a response to phenyl alanine and salicylic acid. *BNRC,* **43**: 1-14.

Farjaminezhad R, Zare N, Zakaria RA and Farjaminezhad M (2013). Establishment and optimization of cell growth in suspension culture of *Papaver bracteatum*: A

biotechnology approach for the baine production. *Turk. J. Biol.*, **37**(6): 689-697.

Fazili MA, Bashir I, Ahmad M, Yaqoob U and Geelani SN (2022). *In vitro* strategies for the enhancement of secondary metabolite production in plants: A review. *BNRC*, **46**(1): 1-12.

Gabr AMM, Arafa NM, El-Ashry AAE and El-Bahr MK (2017). Impact of zeatin and thidiazuron on phenols and flavonoids accumulation in callus cultures of *Gardenia* (*Gardenia jasminoides*). *PJBS*, **20**(7): 328-335.

Halder M, Sarkar S and Jha S (2019). Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. *ELS*, **19**(12): 880-895.

Hendel N, Djamel S, Madani S, Selloum M, Boussakra F, and Driche O (2021). Screening for *in vitro* antioxidant activity and antifungal effect of *Artemisia campestris* L. *Int J Agric Environ Food Sci*, **5**(3): 251-259.

Hussain H, Green IR, Saleem M, Raza ML and Nazir M (2019). Therapeutic potential of iridoid derivatives: Patent review. *Inventions*, **4**(2): 29.

Jeyasri R, Muthuramalingam P, Karthick K, Shin H, Choi, SH and Ramesh M (2023). Methyl jasmonate and salicylic acid as powerful elicitors for enhancing the production of secondary metabolites in medicinal plants: An updated review. *PCTOC*, **153**(3): 447-458.

Karimzadeh F, Haddad R, Garoosi G and Khademian R (2019). Effects of nanoparticles on activity of lignan biosynthesis enzymes in cell suspension culture of *Linum usitatissimum* L. *Russ. J. Plant Physiol.*, **66**: 756-762.

Kong EY, Biddle J, Kalaipandian S and Adkins SW (2023). Coconut callus initiation for cell suspension culture. *Plants*, **12**(4): 968.

Krasteva G (2022). Effect of basal medium on growth and polyphenols accumulation by *Gardenia jasminoides* Ellis cell suspension. *In*: BIO Web of Conferences. *EDP Sci.*, **45**: 02006.

Li Y, Kong D, Fu Y, Sussman MR and Wu H (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *PPB*, **148**: 80-89.

Liu ZB, Chen JG, Yin ZP, Shangguan XC, Peng DY, Lu T and Lin P (2018). Methyl jasmonate and salicylic acid elicitation increase content and yield of chlorogenic acid and its derivatives in *Gardenia jasminoides* cell suspension cultures. *PCTOC*, **134**: 79-93.

Łukowski A, Jagiełło R, Robakowski P, Adamczyk D and Karolewski P (2022). Adaptation of a simple method to determine the total terpenoid content in needles of coniferous trees. *Plant Sci. J.*, **314**: 111090.

Manickam B, Sreedharan R and Elumalai M (2014). 'Genipin' the natural water soluble cross-linking agent and its importance in the modified drug delivery systems: An overview. *Curr. Drug Deliv.*, **11**(1): 139-145.

Mathew R and Sankar PD (2014). Comparison of major secondary metabolites quantified in elicited cell cultures, non-elicited cell cultures, callus cultures and field grown plants of *Ocimum*. *Int. J. Pharm. Pharm. Sci*, **6**(2): 102-106.

Muthukrishnan S, Kumar TS, Gangaprasad A, Maggi F and Rao MV (2018). Phytochemical analysis, antioxidant and antimicrobial activity of wild and *in vitro* derived plants of *Ceropegia thwaitesii* Hook-An endemic species from Western Ghats, India. *JGEB*, **16**(2): 621-630.

Nurmazela V, Ridwanto R and Rani Z (2022). Antioxidant activity test of Barangan Banana Hump's ethanol extract (*Musa Paradisiaca* (L.)) with DPPH (1, 1 Diphenyl-2-Picrylhydrazyl) method. *IJSTM*, **3**(5): 1478-1483.

Olugbami JO, Gbadegesin MA and Odunola OA (2014). *In vitro* evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. *AJMMS*, **43**(Suppl. 1): 101.

Pan Y, Li L, Xiao S, Chen Z, Sarsaiya S, Zhang S and Xu D (2020). Callus growth kinetics and accumulation of secondary metabolites of *Bletilla striata* Rchb. f. using a callus suspension culture. *PLoS One*, **15**(2): e0220084.

Ran D, Hong W, Yan W and Mengdie W (2021). Properties and molecular mechanisms underlying geniposide-mediated therapeutic effects in chronic inflammatory diseases. *J. Ethnopharmacol.*, **273**: 113958.

Reddy YM, Kumar SJ, Saritha KV, Gopal P, Reddy TM and Simal-Gandara J (2021). Phytochemical profiling of methanolic fruit extract of *Gardenia latifolia* Ait. by LC-MS/MS analysis and evaluation of its antioxidant and antimicrobial activity. *Plants*, **10**(3): 545.

Salim SA and Habeeb MS (2018). Elicitation of secondary metabolites production from *Thevetia neriifolia* Juss *in vitro* cultures using chemical elicitors. *ICCABES, (CABES-18).* pp.19-20.

Sankhalkar S and Vernekar V (2016). Quantitative and qualitative analysis of phenolic and flavonoid content in *Moringa oleifera* Lam and *Ocimum tenuiflorum* L. *Pharmacogn. Res.*, **8**(1): 16.

Siddiqui ZH, Mujib A, Abbas ZK, Noorani MS and Khan S (2023). Vinblastine synthesis under the influence of CaCl2 elicitation in embryogenic cell suspension culture of *Catharanthus roseus*. *S. Afr. J. Bot.*, **154**: 319-329.

Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH and Nguyen HC (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *J. Food Qual.*, 1-10.

Umar OB, Ranti LA, Abdulbaki AS, Bola AL, Abdulhamid AK, Biola MR and Victor KO (2021). Stresses in plants: Biotic and abiotic. *CTWR*, pp. 1-8.