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Enhancement of Secondary Metabolite Production in *Tinospora cordifolia* Suspension Cultures Using Gibberellic Acid Elicitation: A Comprehensive Analysis of Alkaloids and Phenols

Ruchi M. Patel[1]
Department of Botany
Government Arts, Commerce & Science College, Limbayat
Surat. India

Khushbu H. Patel[2]
Department of Botany
Government Arts, Commerce & Science College, Limbayat
Surat. India

Meghna R. Adhvaryu[3]
Department of Botany
Government Arts, Commerce & Science College, Limbayat
Surat. India

Abstract:- ***Tinospora cordifolia,*** **a medicinal plant known for its rich bioactive compounds, holds significant pharmacological importance due to its alkaloid and phenolic content. In this study, the effect of gibberellic acid (GA), a plant growth regulator, on the enhancement of total phenols and alkaloids in *in vitro* cell cultures of *T. cordifolia* was investigated. Cells were treated with different concentrations of GA (1–5 mg/L), and the maximum metabolite production was observed at 3 mg/L after 30 days of culture. GA treatment led to a significant increase in total alkaloid content, peaking at 9.454 mg/g DW in the cell extracts, demonstrating GA's role in stimulating alkaloid biosynthesis. Similarly, total phenol content was highest at 3 mg/L GA, with 20.5 mg/g DW in the cell extracts, confirming GA's influence on phenolic pathways. These results highlight GA's potential as an elicitor for enhancing the production of bioactive compounds, offering promising applications for large-scale secondary metabolite production in *Tinospora cordifolia* under controlled conditions.**

Keywords:- *Tinospora cordifolia, Gibberellic Acid, Elicitation, Secondary Metabolites.*

I. INTRODUCTION

The Medicinal plant *Tinospora cordifolia*, commonly known as "Giloy", has significantly deepened our understanding of its pharmacological properties through numerous scholarly studies. These foundational inquiries have established a solid framework for recognizing its therapeutic potential across various health domains. Research highlights the plant's rich array of bioactive constituents with potent antioxidant properties, crucial for reducing oxidative stress and related diseases [1]. *Tinospora cordifolia* has also demonstrated remarkable anticancer properties, attributed to compounds such as berberine, which exhibit non-toxic and potent antitumor effects on cancer cells an insight on the multifarious pharmacological paradigms of a most promising medicinal ayurvedic herb [2]. This herb is particularly esteemed for its immune-boosting capabilities, positioning it as a promising alternative for treating viral infections and other immune-related disorders [3]. Its diverse phytochemical profile, including phenolic compounds, alkaloids, and glycosides, is central to its medicinal attributes [4]. These bioactive compounds have been linked to a wide range of health benefits, from promoting wound healing to managing infections [5].

Traditionally, the production of secondary metabolites in medicinal plants like *Tinospora cordifolia* has relied on conventional methods such as field cultivation and wild harvesting. While these approaches have been widely used, they come with several limitations. The variability in environmental conditions, seasonal fluctuations, and geographical factors can significantly affect the quality and quantity of metabolite production. Additionally, the slow growth rates of medicinal plants and the overharvesting of wild populations pose sustainability concerns. These drawbacks, coupled with the increasing global demand for bioactive compounds, highlight the need for more efficient and controlled methods. As a result, alternative approaches such as *in vitro* cell suspension cultures and elicitation techniques have gained attention for their potential to enhance metabolite production in a sustainable and reproducible manner, providing a viable solution to overcome the limitations of conventional methods.

In vitro suspension culture is increasingly recognized as a sustainable and regulated approach for augmenting the biosynthesis of secondary metabolites derived from medicinal flora. This methodology facilitates the modulation of diverse factors to enhance metabolite productivity, thereby diminishing dependence on indigenous ecosystems. *In vitro*

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cultures furnish a controlled environment, enabling continuous production of secondary metabolites throughout the year, which is essential for fulfilling pharmaceutical requirements [6]. The incorporation of biotic and abiotic elicitors within suspension cultures has been demonstrated to markedly elevate metabolite synthesis, particularly in species possessing established medicinal attributes [7]. Moreover, the application of targeted growth regulators can precisely adjust metabolic pathways, resulting in elevated levels of desired compounds while concurrently reducing the formation of undesired byproducts.

Gibberellins (GAs) are essential phytohormones that control several facets of secondary metabolism and plant growth. They assume a pivotal role in developmental phenomena, encompassing stem elongation, flowering, and fruit maturation, by synthesizing both internal and external stimuli. GAs is indispensable for facilitating stem elongation and flowering across a multitude of plant species, particularly within ornamental horticulture, where they promote bud break and the production of flowering shoots [8]. They participate in the modulation of gene expression pertinent to growth, exhibiting responsiveness to environmental stimuli and nutrient availability, which is critical for optimizing plant architecture and overall yield [9]. GAs interacts with different plant growth regulators, which subsequently impacts the creation of secondary metabolites that are crucial for defence mechanisms in plants and their adaptation to ecological changes [10]. The GA signaling cascade is fundamental to the orchestration of growth and metabolic activities, thereby ensuring that plants are capable of adapting to fluctuating environmental conditions [11]. These intrinsic characteristics of GAs, make it an excellent choice as an elicitor.

On the basis of previous literature Thus, the primary objective of this study is to evaluate the effectiveness of GA in enhancing secondary metabolite production in *Tinospora cordifolia* suspension cultures. The influence of the varying concentrations on the biosynthesis of secondary metabolites, which are crucial for plant defence and overall health, was observed. A range of analytical techniques to assess the changes in metabolite profiles, aiming to identify optimal GA concentrations that enhance the production of total Alkaloids and total phenols in suspension cells. Additionally, the effect of GAs as elicitors was also compared with other elicitors.

II. MATERIALS AND METHOD

- *Source of Explant*

Stems of Tinospora cordifolia were collected from the college botanical garden at Government Science College Vankal, Mangrol, Surat. Gujarat. The explants were used from healthy and disease-free stems.

- *Explant Surface Sterilization and Inoculation*

Nodal stem segments were taken as explants for the callus induction. Subsequent to a thorough washing under continuous tap water for a duration of 30 minutes, the explants were subsequently placed in distilled water. During the process in a laminar flow chamber, they were subjected to surface sterilization with a 2-minute wash of 2% sodium hypochlorite, then treated with 75% ethanol for 3 minutes, and finished with 3 minutes of 0.1% mercuric chloride. Each of these steps was succeeded by a comprehensive rinsing with sterile distilled water. Each explant was placed in sugar tube with MS medium contained 3% sucrose and 3.0 mg/l 2,4-D hormone. All the tubes were incubated at 26 ± ˚C in aseptic conditions.

- *Cell Suspension Culture*

3-4 pieces of pre-established callus tissue (approx. 1 gm each) from the culture tube were transferred using the sterile spoon-headed spatula to conical flasks. Green friable callus was transferred into liquid MS medium with the same sucrose and hormone concentrations, allowing for a more uniform distribution of nutrients and promoting cell division. A light-dark cycle of 16/8 hr. with 25 ± 2°C temperature at 100 rpm was maintained for cell-suspension culture.

- *Addition of GA Different Concentration in Suspension Culture.*

Seven-day-old suspension cultures were filtered using sterile filter paper and a sieve. The cells were gathered with a sterile spatula and placed into a sterile petri plate. A freshly prepared 50 ml liquid medium containing varied concentrations of Gibberellic acid (ranging from 1 to 5 mg/l) is supplemented with precisely measured 2 gm of cellular suspension, with a control group maintained without hormone application. All the flasks were placed in the above-mentioned controlled conditions in order to incubate the cell culture. The cells were finally harvested on the 30th day.

- *Extraction and Preparation of Samples*

After the treatment of elicitors, the cells were filtered and washed several times with distilled water. Suspension was centrifuged at 3000 rpm for 7 min. The supernatant MS medium and cell were dried separately in an oven at 45 °C. 10 ml of methanol was added to the yield and sonicated for 1 hr. Samples are filtered with Wattman filter paper and stored in amber glass bottles and stored in a Refrigerator at 4 °C.

- *Preparation of Standard*

Berberine is considered a standard compound for total alkaloid content and gallic acid is considered a standard compound for total phenol content. For both standards, 1 mg/ml stock solutions in methanol were prepared. Using these stocks, working standards of 5, 10, 20, 40, 60, 80, and 100 µg/ml were made and used in the experiments.

- *Qualitative Test*

Chemical tests for screening and identification of Bioactive compound constituents like Alkaloid, Phytosterol, Flavonoids, Cardiac glycoside, Phenolic compounds, Terpenoids, and Saponins in all samples were conducted using standard protocols to ensure accurate results and reliable identification of these important phytochemicals [12].

- *Estimation of Total Phenol Content*

The Folin-Ciocalteu test was used to estimate the extracts' total phenolic content [13]. Firstly, aliquot 2 ml of the solution derived from the selected plant extracts or the standard into separate 25 ml volumetric flasks. Subsequently,

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introduce 1 ml of Folin-Ciocalteu reagent, pre-diluted with distilled water at a 1:10 (v/v) ratio. along with 1 ml of sodium carbonate into each flask. Following this addition, the reaction mixture was subjected to a vortex for a duration of 15 seconds and allowed to stand for 15 minutes at ordinary temperature to facilitate colour development. Upon completion of the incubation period, the absorbance of the reaction mixture was measured at a wavelength of 765 nm utilizing a spectrophotometer. The quantification of phenolic content in the plant extracts was conducted by reference to the standard curve derived from a series of varying concentrations (5, 10, 20, 40 and 80µg/ml) of Gallic acid. To ensure reproducibility and the acquisition of accurate results, three measurements were obtained for each plant sample. The total phenolic content was expressed as the mg of Gallic acid equivalents per gm dry weight of the plant sample.

- *Detection of total Alkaloid content*

According to [14], total alkaloids were calculated using a spectrophotometer and bromocresol green (BCG) method. A 2 ml sample was put into a separator funnel. Then, 5 ml phosphate buffer (pH 4.7) and 5 ml 10-4 M bromocresol green (BCG) solution were added. After continuous stirring, the mixture was further diluted by adding chloroform in 10 ml volumetric flask. Following the same procedure, various reference standard solutions of berberine (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance was measured at 415 nm using a UV/ Visible spectrophotometer. The alkaloid concentration was represented in mg/g DW of extract for the whole amount.

- *Thin layer Chromatography (TLC) Analysis*

The 3 µl Methanolic extract was applied to pre-coted thin layer chromatography plates (Merk, Silicagel 60 F254) and developed in solvent system Methanol: water: Acetic acid (8:2:0.1).

- *Statistical Analysis*

The mean values of three replicates were derived, and differences between means were analyzed using Tukey's multiple range test. Statistics were run on SPSS. Differences of $p \leq 0.05$ were considered significant.

III. RESULT AND DISCUSSIONS

In the present study, Establishment of callus culture of *Tinospora cordifolia* on hard MS medium was performed using stem as explant from field grown parent plant. Callus initiation was observed after 4 days of inoculation on MS medium enriched with 2-4, D (3.0 mg/L). After 1 week, callus was further introduced to fresh MS medium containing different concentrations of Gibberellic acid.

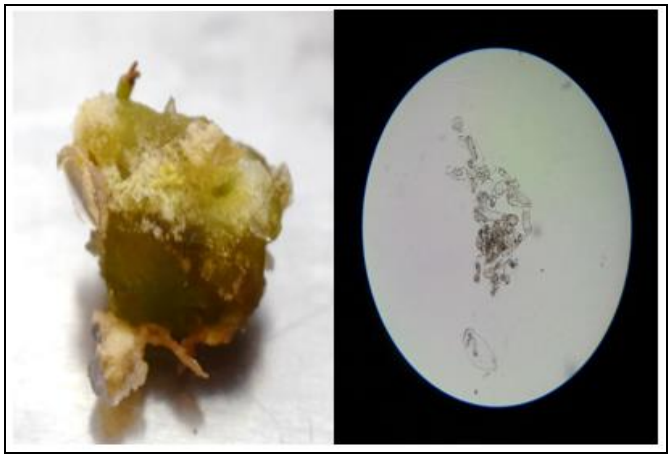

Fig 1 Callus Developed from 3.0 mg/L 2,4-D Supplemented Medium at 5th day, b. Suspension Cells Under Microscope

After observing a peak in alkaloid production around the 25th day of GA treatment, the cells were harvested on the 30th day to ensure maximum metabolite accumulation. Since secondary metabolites like alkaloids, Phenols are primarily produced during the stationary phase, this timing was chosen to capture optimal biosynthesis. The harvested samples underwent qualitative and quantitative assays, confirming a significant increase in alkaloid content in GA-treated cultures compared to the control.

- *Phytochemical Screening*

Phytochemical screening can help identify bioactive agents that may be useful in producing therapeutic drugs or as dietary supplements [15]. By comparing the presence or absence of specific compounds in treated versus untreated samples, we can assess how gibberellic acid (GA) influences metabolic pathways. It also helps in identifying any shifts in secondary metabolite profiles due to hormonal treatment.

All the samples, including the mother plant, *in vitro* cell and MS methanolic extract, were subjected to various chemical tests to detect their phytochemical constituents. As demonstrated in Table 1, the results showed the presence of cardiac glycoside, flavonoids, alkaloids, and phenolic compounds all of which are recognized for potential therapeutic uses in both the cell extract and the MS medium methanolic extract. However, Tannins and cardiac glycosides absent only in MS Medium extract and Terpenoids and Saponins are absent in both the Cellular and extra cellular (Medium) extract. The results indicate that the phytochemicals detected in the medium were either leached out from the cells or released due to cell bursting.

- *Effect of Gibberellic Acid on Tinospora cordifolia Suspension Cultures*

In this study, the effect of Gibberellic acid (GA) on the fresh weight (FW) and dry weight (DW) of cells and MS medium was also analyzed. The FW of cells ranged from 2.302 ± 0.042 g in the control to varying values in the treated samples, with the highest FW recorded at 2 mg/L GA (3.075 ± 0.047 g), followed by a decline at higher concentrations. The DW of the MS medium fluctuated, with a notable decrease at 4 mg/L GA (0.176 ± 0.005 gm), but peaked at 5

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mg/L GA (2.457 ± 0.073 gm). Similarly, the DW of cells showed variation, with the maximum weight at 5 mg/L GA (0.136 ± 0.008 gm), suggesting a concentration-dependent response in biomass accumulation under GA treatment. These results reflect the complex interaction between GA concentration and biomass productivity in *Tinospora cordifolia* suspension cultures.

➢ *Effect of Gibberellic Acid on Total Phenol content*

Total phenol content was determined by the preparation of Gallic acid calibration curve. Gallic acid was taken as standard in different concentration (µg/ml) and measured the absorbance of Gallic acid at 765nm. According to this calibration curve, phenol content of the suspension cell was determined by using following equation:

$Y = 0.0232 X + 0.1235$ ($R^2 = 0.9966$)

Where X is the Gallic acid equivalent (QE) and Y is the absorbance.

In response to both biotic and abiotic stress, plants generate a range of defence mechanisms, including elicitor exposure [16]. This results in the synthesis of many secondary metabolites, including flavonoids, phenolics, and other low molecular weight metabolites [17].

Table 1 Phytochemical analysis for *in vitro* cell and MS media extract with GA hormone treatment at different concentrations

Sr. no	Test	Mother Plant	Control		GA 1.0 mg/L		GA 2.0 mg/L		GA 3.0 mg/L		GA 4.0 mg/L		GA 5.0 mg/L	
			Cell extract	MS media extract	Cell extract	MS media extract	Cell extract	MS media extract	Cell extract	MS media extract	Cell extract	MS media extract	Cell extract	MS media extract
1	Alkaloid	++	++	+	+++	+	+++	+	+++	+	+++	+	+++	+
2	Flavonoid	++	+	+	+	+	++	+	++	+	+	+	+	+
3	Phenol	++	+	+	+	+	+	+	+	+	+	+	+	+
4	Tannins	+	+	-	-	-	+	-	+	-	+	-	+	-
5	Phytosterol	+	++	++	+	++	++	++	++	-	++	++	++	++
6	Cardiac Glycosides	+++	+	-	++	-	++	-	+++	+	+++	-	+	-
7	Terpenoid	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Saponin	-	-	-	-	-	-	-	-	-	-	-	-	-

Preliminary investigations were conducted to ascertain the existence of phytochemicals within the *in vitro* treated suspension extract in comparison to the mother plant. The mark '-' denotes the absence of the identified category of phytochemicals, whereas the marks '+', '++', '+++', and '++++' reveal the varying degrees of detection or existence of the related category of phytochemicals.

Table 2 Effect of gibberellic acid on biomass accumulation in *Tinospora cordifolia* suspension cultures

Sr. no	Gibberellic acid (mg/L)	Fresh weight of cells (gm)	Cell Dry weight (gm)	MS Dry weight (gm)
1	Control	2.302 ± 0.042c	0.092 ± 0.004a	1.710 ± 0.386b
2	1.0	1.534 ± 0.057a	0.088 ± 0.007a	1.544 ± 0.155b
3	2.0	3.075 ± 0.047d	0.124 ± 0.008bc	1.384 ± 0.030b
4	3.0	1.472± 0.041a	0.093 ± 0.004a	1.602 ± 0.025b
5	4.0	1.797 ± 0.038b	0.116 ± 0.006b	0.176 ± 0.005a
6	5.0	1.776 ± 0.085b	0.136 ± 0.008c	2.457 ± 0.073c

Means within a column that share the same letters are not significantly different ($p \leq 0.05$), as determined by Tukey's test.

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The present study evaluated the impact of Gibberellic acid (GA) treatment on the total phenol content in cell suspension cultures of *Tinospora cordifolia*. The total phenol content was measured in both cell extracts and MS medium extracts, with six groups: a control (no GA) and five treatment groups with GA concentrations of 1 to 5 mg/L. (Table 3)

The mother plant exhibited a total phenol content of 2.059 mg/g DW. In the *in vitro* cultured cells, the control group showed a significantly higher phenol content of 18.009 mg/g DW compared to the mother plant. GA treatment resulted in variable effects on phenol levels within the cells. The phenol content was 15.23 mg/g DW at 1 mg/L GA and 14.632 mg/g DW at 2 mg/L GA, showing a slight decrease compared to the control. A peak phenol content of 20.5 mg/g DW was observed at 3 mg/L GA, indicating the highest induction among the treated groups. However, further increases in GA concentration led to a reduction in phenol content: 14.145 mg/g DW at 4 mg/L and 12.03 mg/g DW at 5 mg/L, suggesting a diminishing response at higher GA levels.

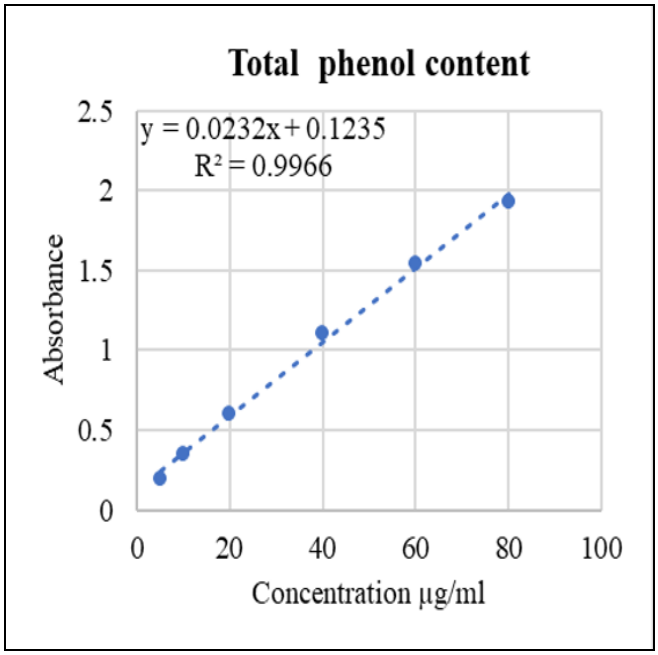

Fig 2 Standard Curve for Phenol (Gallic Acid)

Table 3 Total Phenol content in cellular and extracellular (MS) samples and Mother plant extract.

Sr No.	Sample	Cell extract (mg/g DW)	MS extract (mg/g DW)
1	Mother plant	2.079 ± 0.437ab	
2	Control	20.109 ± 2.485cd	1.059 ± 0.434ab
3	GA (1.0 mg/L)	16.330 ± 3.618bc	1.451 ± 0.229ab
4	GA (2.0 mg/L)	15.832 ± 2.365bc	1.552 ± 0.060b
5	GA (3.0 mg/L)	21.800 ± 3.379d	1.255 ± 0.366ab
6	GA (4.0 mg/L)	15.165 ± 1.358bc	6.065 ± 0.874c
7	GA (5.0 mg/L)	13.130 ± 1.676b	0.453 ± 0.062a

Means within a column that share the same letters are not significantly different ($p \leq 0.05$), as determined by Tukey's test.

The MS medium extracts contained lower phenol levels compared to the cell extracts, with the control group showing 1.039 mg/g DW. The GA-treated medium exhibited phenol contents of 1.051, 1.532, 1.225, 6.035, and 0.423 mg/g DW for 1, 2, 3, 4, and 5 mg/L GA, respectively. Notably, a substantial increase to 6.035 mg/g DW was observed at 4 mg/L GA, indicating an elevated release or secretion of phenolic compounds into the medium at this concentration. Conversely, the lowest phenol content in the medium was recorded at 5 mg/L GA (0.423 mg/g DW), aligning with the observed trend of declining phenol synthesis or secretion at higher GA concentrations. These findings suggest that GA has a concentration-dependent effect on phenol production in *Tinospora cordifolia*, with optimal phenol enhancement observed at intermediate concentrations (particularly 3 mg/L GA for cells and 4 mg/L GA for the medium). Higher GA concentrations appear to negatively impact phenol content, indicating a threshold beyond which the elicitation effect diminishes. In a study on *Stevia rebaudiana*, the application of 2.0 mg/L gibberellic acid (GA3) in adventitious root cultures resulted in the highest total phenolic production (TPP), reaching 147.6 mg/L, demonstrating the potential of GA3 for enhancing secondary metabolite production in plant suspension cultures [18].

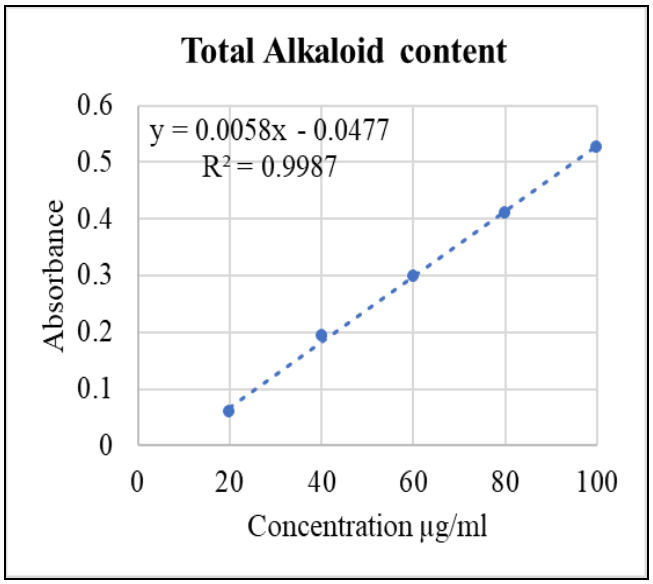

Fig 2 Standard Curve for Alkaloid (Berberine)

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➢ *Effect of Gibberellic Acid on Total Alkaloid Content*

This study investigated the influence of gibberellic acid (GA) as an elicitor on the increase of total alkaloid production in *Tinospora cordifolia* cell suspension cultures. The total alkaloid content was determined and expressed in mg/g dry weight (DW) after the cells and the MS media were removed independently. (Table 4).

Table 4 Total Alkaloid content in cellular and extracellular (MS) samples and Mother plant extract.

Sr. No.	Sample (mg/L)	Cell extract (mg/g DW)	MS extract (mg/g DW)
1	Mother plant	2.409 ± 0.001ab	
2	Control	5.838 ± 0.221c	0.175 ± 0.035a
3	GA 1.0	5.898 ± 0.310c	0.222 ± 0.012a
4	GA 2.0	6.456 ± 0.126d	0.311 ± 0.007a
5	GA 3.0	9.457 ± 0.316f	0.219 ± 0.007a
6	GA 4.0	8.177 ± 0.172e	1.760 ± 0.341c
7	GA 5.0	5.362 ± 0.106b	0.244 ± 0.010a

Means within a column that share the same letters are not significantly different ($p \leq 0.05$), as determined by Tukey's test

In the mother plant, the Total alkaloid concentration was quantified to be 2.409 ± 0.001 mg/g DW. In the context of the *in vitro* cultured cellular extract, the control (without of GA administration) demonstrated an alkaloid concentration of 5.838 ± 0.221 mg/g DW. GA caused a considerable increase in alkaloid synthesis, which was measured at 5.898 ± 0.310 mg/g DW for 1 mg/L GA, 6.456 ± 0.126 mg/g DW for 2 mg/L GA, and 9.457 ± 0.316 mg/g DW for 3 mg/L GA, showcasing the apex of recorded results. At a concentration of 4 mg/L GA, the alkaloid levels showed a small decline to 8.177 ± 0.172 mg/g DW, and then at 5 mg/L GA, it decreased further to 5.362 ± 0.106b mg/g DW, hinting at a potential inhibitory influence of higher GA concentrations. The presence of alkaloids within the MS medium was also identified, although at significantly lower concentrations than in the cellular systems. The control medium revealed an alkaloid concentration of 0.175 ± 0.035 mg/g DW. The medium treated with GA showed levels of 0.222± 0.012, 0.311± 0.007, 0.219± 0.007, 1.76± 0.341, and 0.244± 0.010 mg/g DW corresponding to 1, 2, 3, 4, and 5 mg/L GA, respectively, peaking at a concentration of 4 mg/L GA. These results reveal that GA has a strong effect on enhancing alkaloid production in *Tinospora cordifolia* cell cultures, particularly evident at 3 mg/L GA for the cells and at 4 mg/L GA for the medium. However, at elevated concentrations, GA appears to exert an inhibitory influence, suggesting the existence of an optimal range for elicitation to optimize alkaloid yield. the study examines by Ali et. al., [19] shown the impact of methyl jasmonate, jasmonic acid, and gibberellic acid on *Artemisia absinthium* L. cell suspension cultures, revealing inhibition in dry biomass accumulation, shorter growth phases, enhanced phenolic and flavonoid content.

In the current study, the application of gibberellic acid (GA) to *in vitro* cultures of *Tinospora cordifolia* significantly enhanced alkaloid production, confirming GA's efficacy as an elicitor. This is the first report highlighting the role of GA in boosting secondary metabolite pathways, particularly alkaloid biosynthesis, in this plant. The results demonstrate a clear dose-dependent relationship between GA treatment and increased.

Alkaloid yields, with optimal results observed at 3 mg/L of GA. These findings suggest a promising approach for large-scale production of bioactive compounds in controlled environments. Similarly, total phenol content also peaked at 3 mg/L GA, although the overall trend was more variable across concentrations. This indicates that GA not only promotes alkaloid biosynthesis but also influences the accumulation of phenolic compounds, which are essential for the plant's defense mechanisms and antioxidant activities. These findings suggest that GA acts as an effective elicitor, enhancing the production of key secondary metabolites in *in vitro* conditions.

➢ *Thin Layer Chromatography (TLC)*

For the TLC analysis of GA-treated samples, six distinct spots were detected, each representing different compounds. The reference standard berberine exhibited an Rf value of 0.67. The control and GA-treated samples (1 to 5 mg/L concentrations) showed spots at Rf values of 0.41, 0.63, 0.67 (matching the berberine standard), 0.77, 0.87, and 0.35. While all the spots appeared in every sample, their intensity varied depending on the GA concentration. This suggests that the GA treatment influenced the production or accumulation of these compounds, potentially enhancing the intensity of some at specific concentrations.

This study presents the first report on the enhancement of alkaloid production using gibberellic acid (GA) in *in vitro* cultures. While GA's influence on various secondary metabolites like flavonoids and phenolics has been previously documented, its role in stimulating alkaloid biosynthesis remains largely unexplored. This research bridges this gap, demonstrating that GA can significantly boost alkaloids accumulation in cultured plant cells, offering new insights into its application in secondary metabolite production.

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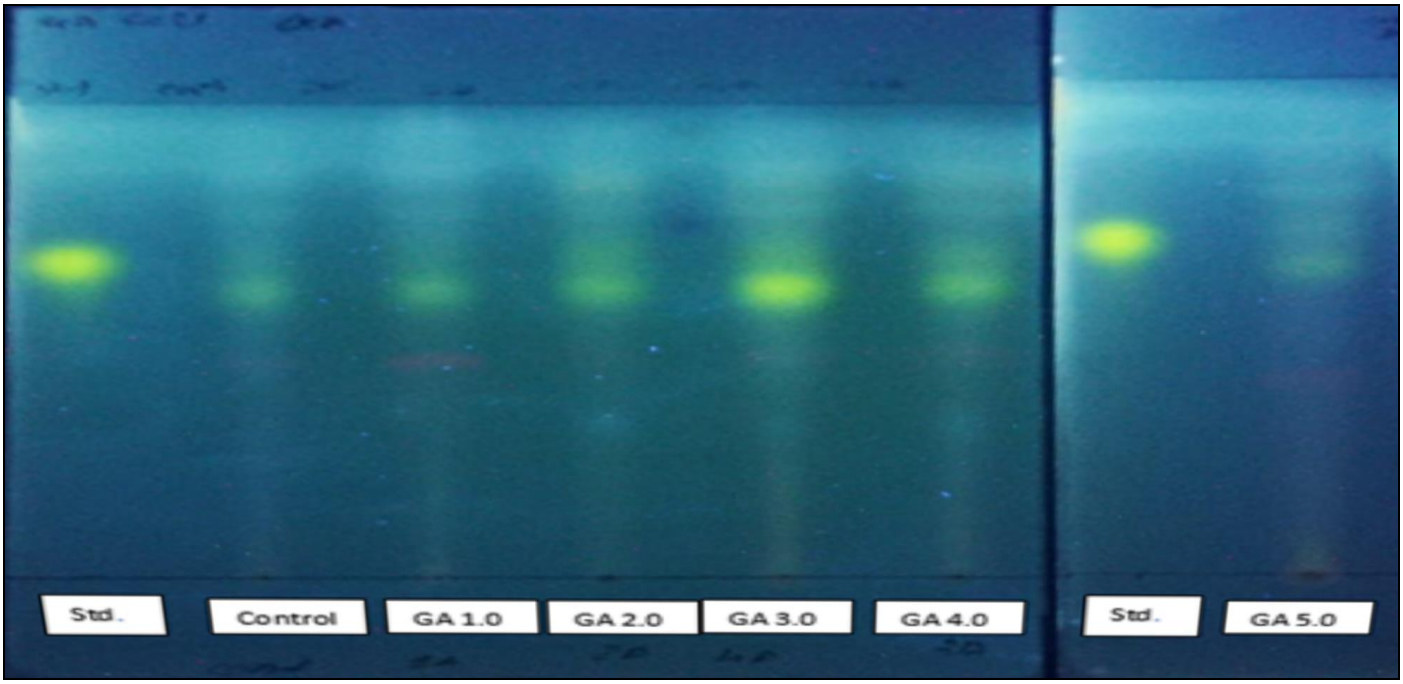

Fig 3 TLC profile of gibberellic acid treated cell culture samples of *Tinospora cordifolia.*

IV. CONCLUSION

The study demonstrated that Gibberellic acid (GA) effectively enhances the production of total alkaloids and phenols in *Tinospora cordifolia* suspension cultures, with optimal results observed at specific GA concentrations. For alkaloids, the highest enhancement occurred at 3 mg/L GA in cells, while 4 mg/L GA significantly increased phenol levels in the MS medium. However, higher GA concentrations led to a decline in both alkaloid and phenol content, suggesting an inhibitory effect beyond certain thresholds. These findings highlight the potential of GA as san elicitor to boosst secondary metabolite production in *Tinospora cordifolia*, with careful optimization of concentration being crucial for maximizing yield.

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