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Effect of Plant Growth Regulators (PGRs) on Biomass and Flavonoid Production of *Talinum paniculatum* Callus Culture

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Abstract. *Talinum paniculatum Jacq (Gaertn), locally known as Javanese Ginseng, is an essential medicinal plant characterized by high flavonoid content with antioxidant and antimicrobial activities. Compared to other natural sources, a larger number of T. paniculatum leaves is required to obtain high amounts of flavonoid for drug development. In this context, in vitro culture can be used to increase flavonoid production in a controlled condition without reducing plant population. The concentration of Plant Growth Regulators (PGRs) is capable of influencing biomass and flavonoid accumulation in callus culture from several species. Despite these benefits, there is limited information about the optimal concentration of PGRs for flavonoid production from T. paniculatum callus culture. Therefore, this study aimed to investigate the optimum concentration of 2,4-D and kinetin for callus growth, as well as flavonoid production from T. paniculatum. The investigation was carried out using a completely randomized design (CRD), where young leaves explants were cultured on MS media supplemented with various concentrations of 2,4-D (0, 0.5, 1, 2) mg/L and kinetin (0, 0.5, 1, 2) mg/L for callus and flavonoid production. The results showed that the highest callus biomass of 0.105 g was produced from MS medium with 2 mg/L of 2,4-D and 1 mg/L of kinetin. Meanwhile, the highest total flavonoid content of 25.66 mg QE/g DW was produced from media supplemented with 1 mg/L of 2,4-D and 2 mg/L of kinetin. These results showed that different combinations of 2,4-D and kinetin were required to increase biomass and flavonoid production. In conclusion, this study provided valuable information for the development of bioactive compounds through in vitro culture.*

Keywords: *Biomass, callus, 2,4-D, Talinum paniculatum, flavonoid, kinetin.*

Citation
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INTRODUCTION

Talinum paniculatum Jacq.(Gaertn.) is a herbaceous plant belonging to the *Talinaceae* family that is widely distributed in Asia. In Indonesia, *T. paniculatum* is locally called Javanese Ginseng due to the high similarity of bulging root morphology with Korean Ginseng (*Panax ginseng*) (Ahmad & Anggita, 2019). This plant is widely used as a raw material in herbal medicine, vegetable consumption, and ornamental plant (Liu et al., 2018). Currently, Javanese ginseng is becoming popular as a substitute for Korean Ginseng in herbal medicine due to its relatively lower price, offering a cost-effective alternative (Sulistiono, 2017). *T. paniculatum* has several health benefits, such as anti-diarrhea, anti-inflammation, reduced cough, aphrodisiac, tonic, and increased breast milk (Setyowati & Setyani, 2019). The medicinal benefits are associated with secondary metabolites contained in all parts, such as saponin, flavonoid, tannin, alkaloid, and sterol content (Lakitan et al., 2021; Manuhara et al., 2015).

Flavonoid is produced in *T. paniculatum*, particularly in roots and leaves, characterized by high antioxidant (Menezes et al., 2021), antimicrobial and antifungal bioactivities (Cerdeira et al., 2020). Although flavonoid can be extracted directly from all parts of plant, the sustainable production is influenced by nutrition status, season, developmental stages, environmental stress, and genetic factors. Furthermore, increasing demands for flavonoid from *T. paniculatum* in pharmaceutical industries have posed significant concerns for the future existence of the species (Pramita et al., 2018; Wawrosch & Zotchev, 2021). To overcome these challenges, callus culture presents a biotechnological method with promising potential. Production of secondary metabolites through callus culture has several advantages, including the generation of explants rapidly within a relatively short period (Bhojwani & Dantu, 2013; Efferth, 2019; Habibah et al., 2016). According to Jamwal et al. (2018), Plant Growth Regulators (PGRs) are commonly used to induce the synthesis of secondary metabolites in plants. These regulators play a significant role in the regulation of antioxidant potential, fundamental growth, developmental processes, and generation of plant secondary metabolites in plant tissue culture. The response of plants to PGRs varies based on species, age, varieties, environmental factors, stage of development, physiological and nutritional status, as well as endogenous hormonal balance. Several studies have been conducted and validated on a large-scale plant scale to investigate the impact of various PGRs on the synthesis of secondary metabolites (Sari et al., 2018; Park et al., 2020; Ramabulana et al., 2021; Kim et al., 2023).

Generally, PGRs regulate growth of callus and modulate the accumulation of secondary metabolites including flavonoid by modulating gene expression (Habibah et al., 2016; Ramirez-Estrada et al., 2016; Thakur et al., 2019). Among various types of PGRs, 2,4-D is a synthetic auxin frequently used in the in vitro culture to induce callus formation (Sari et al., 2018). According to Tan et al.(2010), the supplementation of 2,4-D in MS media is more effective in producing optimum callus growth from *Centella asiatica* leaves explant compared to other auxin groups such as picloram, NAA, IBA, and dicamba. The supplementation of cytokinin hormone in the media along with auxin at the proper concentration could stimulate callus growth. Among the commonly used cytokines, kinetin is often used to induce callus culture, which produces higher biomass and a faster growth rate when combined with 2,4-D (Sari et al., 2018; Tan et al., 2010). These PGRs combinations are essential to increase callus biomass that can further synthesize flavonoid.

Several studies have been carried out focusing on the optimization of PGRs on flavonoid production through callus culture from medicinal plants. Habibah et al.(2021) reported that the addition of 1 mg/L of 2,4-D and kinetin in the MS media could produce the highest total flavonoid content in *Dioscorea esculenta* callus culture under light conditions. Furthermore, the combination

of 2 mg/L of 2,4-D and 0.5 mg/L of BAP in the MS media was observed to significantly increase the total flavonoid content (TFC) (6.20 mg/g DW) of Centella asiatica callus culture (Rao et al., 2015). Previous reports also showed that the combination of 1.5 mg/L 2,4-D and 1.5 mg/L kinetin produced optimum *T. paniculatum* callus biomass and saponin production (Wardani et al., 2004). However, there is no information on the optimization of PGRs concentration on callus growth and flavonoid production from *T. paniculatum* callus culture. Therefore, this study aimed to investigate the optimum concentration of 2,4-D and kinetin on callus growth with flavonoid production from T. *paniculatum*. The results are expected to provide valuable information regarding the optimal production of flavonoid from callus culture of *T. paniculatum*.

MATERIALS AND METHODS

Plant Material

The study was conducted from March to June 2022 at the Basic Biotechnology Laboratory, Faculty of Biotechnology, Universitas Kristen Duta Wacana, Yogyakarta. The investigation was carried out using an experimental laboratory method consisting of callus induction, biomass and flavonoid production, callus morphology, biomass, and total flavonoid content analyses. The mother plant of *T. paniculatum* used was obtained from Merapi Farma Herbal, Harjobinangun, Pakem, Yogyakarta, in February 2021. Subsequently, adequate maintenance was performed at the Biotechnology Laboratory, Universitas Kristen Duta Wacana, while identification was performed by Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta.

Explant and Medium Preparation

The explants used in this study were young and healthy leaves of parent plant collected at positions 2-3 of the apical shoot. Callus culture medium used was Murashige and Skoog (MS) medium (Sigma), while MS medium was supplemented with myo-inositol (Merck), 30 g/L sucrose (Merck), and 0.8% (w/v) agar (Oxoid). Growth regulators, including 2,4-D (BDH) at various concentrations (0, 0.5, 1, 2) mg/L and kinetin (Sigma Aldrich) at (0, 0.5, 1, 2) mg/L were used to induce callus and flavonoid production. Subsequently, pH of the media was adjusted to 5.7 - 5.8, followed by sterilization using an autoclave (Hirayama Manufacturing Corporation) at 121°C 1 atm for 30 minutes.

Establishment of Callus Culture

The sterilization of explants was carried out in a laminar air flow (LAF). Initially, leaves were washed with running tap water and immersed in liquid detergent added with 3 drops of Tween 80 (Merck) for 45 seconds, followed by rinsing three times using sterile distilled water. In LAF, leaves explants were sterilized using 70% alcohol for 3 minutes and rinsed with sterile distilled water three times. The sterile leaves were cut aseptically (1 cm x 1 cm) and inoculated on MS medium (Murashige & Skoog, 1962), supplemented with various combinations of 2,4-D and kinetin concentrations with slight modification, as shown in Table 1 (Tan et al., 2010). Callus culture was maintained in a chamber at 25°C and 3000 lux light intensity for 24 hours (Natasha & Restiani, 2019).

Callus Growth and Biomass Measurement

The observation of cultivated explants was conducted every 2 days to determine growth and morphology of callus. After 28 days of culture, callus were harvested and oven-dried at 40°C to achieve a constant dry weight. Subsequently, dried callus was weighed and recorded as biomass of callus (DW), followed by morphology observation using a stereo microscope with 40x magnification (Wijaya et al., 2020). The identification of callus texture (friable and compact) and color (white, green, yellow, and black) was based on Noor Aini Habibah et al.(2023) and Sari et al.(2018).

Table 1. Various treatments of Kinetin and 2,4-D in MS solid medium

Kinetin concentration (mg/L)(K)	2,4-D concentration (mg/L) (D)			
	0	0.5	1	2
0	K_0D_0	K_0D_{05}	K_0D_1	K_0D_2
0.5	$K_{0.5}D_0$	$K_{05}D_{0.5}$	$K_{0.5}D_1$	$K_{0.5}D_2$
1	K_1D_0	$K_1D_{0.5}$	K_1D_1	K_1D_2
2	K_2D_0	$K_2D_{0.5}$	K_2D_1	K_2D_2

Total Flavonoid Extraction and Determination

In this study, dried callus was ground to a powder using a pestle and mortar. Initially, 5.0 g of filtered callus powder was extracted in 50 mL methanol 96% (Merck) for 24 hours at room temperature. The extract was filtered and re-extracted using methanol 96% 24 hours at room temperature, which was allowed to evaporate using a water bath at a temperature of 65°C to achieve a final volume of 1 mL. TFC was determined using the procedure of (Kim et al., 2003) with slight modifications. During this process, 0.1 mL of aliquot sample was dissolved in methanol 96% to obtain a total volume of 1 mL. The sample was mixed with 4 ml distilled water, 0.3 mL $NaNO_2$ 5%, and incubated for 5 minutes of incubation. Subsequently, the solution sample was added with 0.3 mL $AlCl_3$ 10% and incubated at room temperature for 6 minutes, followed by the addition of 2 mL of NaOH 1 mol/L. Finally, the distilled water was added to the mixture to achieve a total volume of 10 mL, followed by thorough dissolution and incubation for 15 minutes. The absorbance of the mixture sample was determined at 510 nm, where quercetin was used as a standard to calculate TFC. Based on the calculation, TFC was measured as mg quercetin equivalent per g of sample dry weight (mg QE/g DW).

Data Analysis

The results of data analysis, including the percentage of callus formation, initiation time, biomass, and TFC from various treatments, were presented as mean ± standard deviation from five replicates. The analysis was performed using ANOVA (SPSS 25, Inc., USA), followed by Duncan Multiple Range Test (DMRT) post hoc test to evaluate significant differences at ($P<0.05$). The results of callus percentage, initiation time, and morphology were presented in tables, while callus biomass and TFC were visualized in histogram

RESULTS AND DISCUSSION

Callus Growth

The results showed that all MS medium added with different concentrations of 2,4-D and kinetin could induce callus growth from leaves explants (100 %). However, media without PGRs (control) showed callus induction of only 50%, which occurred 6 days after inoculation, as presented in Table 2. The best medium that resulted in the fastest callus induction 4 days after inoculation (DAI) was the combination of 2,4-D concentration ranging from 0.5 to 2 mg/L and kinetin at 1–2 mg/L. Meanwhile, the combination of 1 mg/L 2,4-D and 0.5 mg/L kinetin in MS medium caused slower callus formation (6.4 ± 1.67 days). These results showed that the optimum ratio of 2,4-D (auxin) and kinetin (cytokinin) in the MS medium significantly influenced callus induction.

The addition of various concentrations of 2,4-D and kinetin into MS media affected

callus initiation time. In K_0D_2, K_1D_1, K_1D_2, and $K_2D_{0.5}$ treatments, callus initiation time was relatively faster compared to others, which occurred on 4 DAI. These results showed that a greater increase in 2,4-D concentration in MS medium had effect on callus growth speed from leaves explants. Similarly, Tan et al. (2010) reported that a high concentration of 2,4-D in MS media increased the formation of Centella asiatica callus (70%) compared to the control. This phenomenon occurred due to the higher use of 2,4-D in combination with cytokinin at a certain ratio, which could stimulate callus growth. An increase in cell water permeability and osmotic pressure, along with a decrease in pressure, an enlargement of the cell wall, high protein synthesis, and elevated plasticity, were caused by the ability of 2,4-D in auxin group. The addition of auxin hormone to the media could cause plasticity and loosening of cell walls. This is because auxin releases H^+ into the cell wall and lowers the pH, causing the cell wall to loosen and growth formation. Furthermore, the addition of cytokinin to media increases cell division, facilitating the interaction between PGRs group to enhance callus initiation time (Tan et al., 2010; Habibah et al., 2016; Sari et al., 2018).

Table 2. Effect of 2.4-D and kinetin concentration on callus formation percentage and callus initition time from *T. paniculatum* leaves explants

PGRs		Callus Formation (%)	Callus Initiation Time (Days)
2.4-D (mg/L)	Kinetin (mg/L)		
0	0	50	6.0 ± 0.00^{cde}
0.5	0	100	4.4 ± 0.89^{ab}
1	0	100	4.4 ± 0.89^{ab}
2	0	100	4.0 ± 0.00^{a}
0.5	0.5	100	5.6 ± 0.89^{bcd}
1	0.5	100	6.4 ± 1.67^{de}
2	0.5	100	6.0 ± 1.41^{cde}
0.5	1	100	4.4 ± 0.89^{ab}
1	1	100	4.0 ± 0.00^{a}
2	1	100	4.0 ± 0.00^{a}
0.5	2	100	4.0 ± 0.00^{a}
1	2	100	4.4 ± 0.89^{ab}
2	2	100	4.8 ± 1.10^{abc}

Callus Morphology

The addition of 2,4-D and kinetin concentrations affected the percentage of callus formation, enhancing morphology based on texture and color, as shown in Table 3. Subsequently, texture observation was carried out to determine callus quality, showing whether cell division was active or died through visual appearance. Treatment with the addition of different concentrations of 2,4-D resulted in friable callus. Similarly, Tan et al.(2010) produced friable callus from MS media supplemented with different concentrations of 2,4-D. The addition of kinetin at various concentrations caused callus texture to become compact and intermediate. These results showed that different types and concentrations of growth regulators

could cause variation in callus texture, as reported by Habibah et al.(2023). However, Sari et al.(2018) stated that increasing the concentration of 2,4-D and kinetin produced friable callus.

The addition of 2,4-D and kinetin concentrations affected various callus color changes in *T. paniculatum* leaves explants, as shown in Table 3. The result showed that callus from all treatments were white, greenish-yellow, pale green, and brownish-yellow. Specifically, white callus showed the absence of meristematic conditions and plastid differentiation. The pale green color showed that chlorophyll started to form in callus cells caused by the process of photosynthesis as a reaction to lighting on the chloroplasts. The greenish-yellow color of callus showed an active division of young cells into maturity as well as the interaction between auxin and cytokinin. In this process, kinetin plays a role in stimulating the formation of chlorophyll, while auxin inhibits chlorophyll. The brown color showed the accumulation and oxidation of phenolic compounds in callus (Habibah et al., 2023; Sari et al., 2018). According to Harahap et al.(2020), the ideal callus color is greenish white, showing the presence of chlorophyll pigment and active division, while brownish has bad quality. Sari et al.(2018) stated that the best texture quality was friable callus, showing the regeneration capacity of callus to form shoot and root. Meanwhile, compact callus was observed to have a capacity to produce secondary metabolites. These results showed that the addition of 2,4-D and kinetin at higher concentrations of 2 mg/L caused callus to turn brownish-yellow. Similarly, (Habibah et al.(2023) and Harahap et al.(2020) reported brownish-white to yellow callus from MS media supplemented with the increasing concentration of 2,4-D and kinetin.

Mahadi and Sari (2016) stated that the addition of cytokinin into MS media played an important role in the transportation of nutrients, which was capable of causing the lignification process to produce a harder and more compact texture. Moreover, the formation of compact callus is attributed to the interaction between endogenous hormones and exogenous PGRs concentration added to MS media, particularly auxins, and cytokinins. Nutrients in the media can also diffuse into the explants because cell walls are loose and flexible due to the influence of auxin. This process is continued until the cell becomes turgid, where water potential and osmotic potential have been balanced. The addition of cytokinins affects cell division and differentiation, thereby accelerating cell and callus formation with a compact texture (Ulva et al., 2011). Friable callus has a soft texture, facilitating easy separation, and suitability for use as a cell suspension (Mahadi & Sari, 2016). Meanwhile, compact callus is used for production of secondary metabolites due to the ability to accumulate more metabolites.

Callus Biomass

Treatment with the addition of 2,4-D and kinetin with different concentrations resulted in varied callus biomass, as shown in Figure 1. Based on the results, the highest average callus biomass of 0.105 g DW was found in 1 mg/L kinetin and 2 mg/L 2,4-D. This was followed by 1 mg/mL kinetin 2 mg/L 2,4-D, with an average callus biomass of 0.095 g, showing that increased concentration was triggered by the addition of balanced PGRs. Similarly, the addition of PGRs concentrations (kinetin and 2,4-D) at the same ratio produced insignificantly different results, including 0.5:0.5, 1:1, and 2:2, with an average callus biomass of 0.086 g, 0.089 g, and 0.086 g. These results showed that the increase in callus dry weight could be obtained through the addition of a balanced PGRs concentration.

Growth of callus was determined through biomass, where the result showed

that a high concentration of 2,4-D without kinetin increased callus biomass. This showed that the addition of 2,4-D concentration was directly proportional to the increase in biomass. Rahayu et al.(2003) reported that leaves explants of *Acalypha indica* L. were inoculated into MS medium with the addition of 2,4-D with a concentration of 0 – 3 mg/L. Based on these results, callus biomass was observed to increase with the addition of a higher 2,4-D concentration. Callus growth can be stimulated through the addition of 2,4-D (auxin hormone) concentration, as shown by the larger size and irreversible increase in callus biomass. Restiani et al. (2022) stated that callus culture of *T. paniculatum* leaves explants were inoculated into MS media with the addition of 2,4-D and kinetin at various concentrations.

The lower concentration of kinetin resulted in relatively reduced callus biomass, showing the need for high kinetin. According to Wardani et al.(2004), the addition of kinetin to MS media caused a significantly different effect on increasing callus biomass, with 2,4-D showing high effectiveness. Optimum callus biomass was influenced by the balance concentration of 2,4-D (auxin) and kinetin (cytokinine). This is attributed to the balance concentration between endogenous hormone and exogenous hormone from explants, which stimulates the activation of genes and transcription factors responsible for cell proliferation at G_1 to S phase (Habibah et al., 2023). Based on the results, an increase in callus biomass was obtained through the addition of the balanced PGRs concentration. Wardani et al.(2004) stated that callus and biomass growth could be stimulated through kinetin and 2,4-D, as shown by the highest dry weight of 414.60 mg at a concentration of 1.5 mg/L 2,4-D and 1.5 mg/L kinetin. The speed of cells in dividing and multiplying, as well as cell enlargement, are factors that affect the resulting callus biomass. Furthermore, the addition of a combination of auxin and cytokinin (2,4-D and kinetin) into MS media could affect the speed of cell proliferation. This suggested that PGRs significantly influenced RNA metabolism in protein synthesis as a source of energy during the transcription process of RNA molecules. Consequently, a significant increase in protein synthesis during growth could increase callus biomass (Habibah et al., 2023).

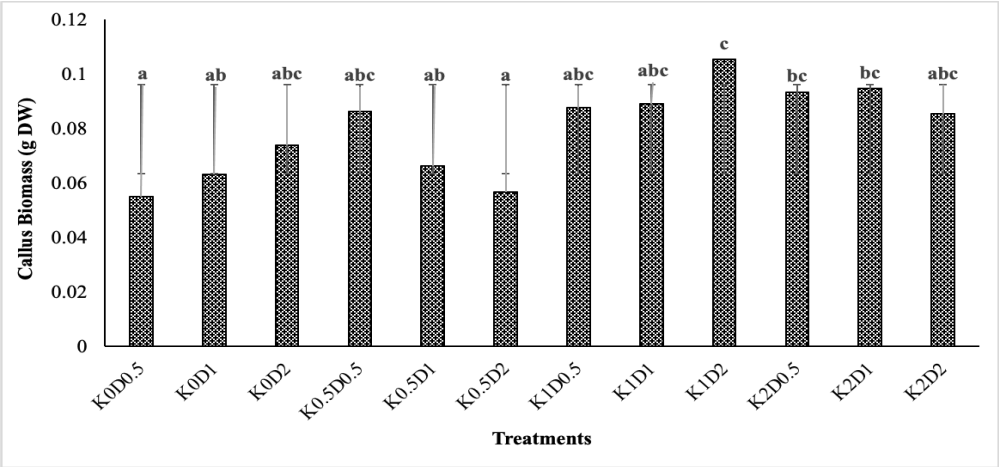

Figure 1. Effect of 2,4-D and kinetin concentration on *T. paniculatum* callus biomass at 28 days of culture
*) The bar shows standard error of mean (n = 5). Different letters showing significant differences (P ≤ 0.05)

Table 3. Effect of 2,4-D and kinetin concentration on callus morphology from *T. paniculatum* leaves explants

PGRs		Callus Morphology		Figure
2,4-D (mg/L)	Kinetin (mg/L)	Color	Texture	
0	0	White	Friable	
0.5	0	Yellowish Green	Friable	
1	0	Yellowish Green	Friable	
2	0	Yellowish White	Friable	
0.5	0.5	Brownish Yellow	Intermediate	
1	0.5	Brownish Yellow	Intermediate	

PGRs		Callus morphology		Figure
2,4-D (mg/L)	Kinetin (mg/L)	Color	Texture	
2	0.5	Brownish Yellow	Intermediate	
0.5	1	Pale Green	Compact	
1	1	Pale Green	Compact	
2	1	Pale Green	Compact	
0.5	2	Pale Green	Compact	
1	2	Pale Green	Compact	
2	2	Brownish Yellow	Compact	

TFC in Callus Culture

Flavonoid is a polyphenolic compound characterized by low molecular weights with biological activity as antioxidants. The measurement of TFC was carried out to determine the effect of 2,4-D and kinetin concentration on TFC in *T. paniculatum* callus. Based on the results presented in Figure 2, the addition of a concentration of 2,4-D (0.5 – 2 mg/L) without kinetin resulted in varying total flavonoid levels. The highest average TFC was found at a concentration of 1 mg/L 2,4-D, which was 11.99 ± 0.39 mg (QE)/g DW, while the lowest value of 9.59 ± 1.11 mg (QE)/g DW was obtained at 0.5 mg/L kinetin. Although the addition of 2,4-D to MS media could stimulate cell division and callus growth, increasing the concentration without kinetin did not produce high levels of total flavonoid. Treatment with the addition of 2,4-D and kinetin at various concentrations resulted in an average variation of total flavonoid. Based on Figure 2, the highest average total flavonoid production of 25.66 ± 0.98 mg (QE)/g DW was obtained from 2 mg/L kinetin concentration and 1 mg/L 2,4-D 1 mg/L, while the lowest value of 8.55±0.30 mg (QE)/g was achieved from 0.5 mg/L kinetin and 2,4-D 2 mg/L. The addition of kinetin with a higher concentration compared to 2,4-D showed higher total flavonoid production compared to other treatments. This result showed that the addition of kinetin at higher concentrations led to greater total flavonoid levels on callus culture of *T. paniculatum* leaves explants.

The results presented in Table 3 and Figure 2 showed that callus texture influenced the TFC. In callus with compact and friable texture, the value of TFC was higher compared to the sample with intermediate texture. Based on the results, callus treated with various kinetin and 2,4-D ratios, including 2:1 mg/L, 2:0.5 mg/L, 1:0.5 mg/L, and 1:1 mg/L, showed a compact texture, while 0.5:2 mg/L had an intermediate texture. Jamwal et al.(2018) reported that PGRs could regulate production of secondary metabolites through in vitro culture. Similarly, this study showed that the addition of kinetin at a higher concentration than 2,4-D produced greater total flavonoid compared to other treatments. These results suggested that the addition of kinetin with a higher concentration produced optimal TFC in callus culture from T. paniculatum leaves explants. According to Ravanfar et al. (2020), the addition of 2 mg/L zeatin (cytokinin) to the shoot culture media of red cabbage explants resulted in higher total phenolic and flavonoid content compared to control, which was 5.18 mg gallic acid equivalent/g DW and 1.52 mg gallic acid equivalent/g DW. Furthermore, the addition of zeatin levels in the media influenced the accumulation of phenolic and flavonoid levels produced. The increase in various phenolic compounds and flavonoid occurred due to a high concentration of zeatin to 2 mg/L. Sayd & Shakour (2014) reported that the addition of 2 mg/L 6-Benzyladenine (BA), in the same hormone group with zeatin and kinetin, resulted in the highest flavonoid content of 24.41 mg/g DW and phenolic of 67.91 mg/g DW compared to other BA treatments from shoot culture of *Cephalotaxus harringtonia* L. Therefore, the addition of exogenous cytokinin group at a certain concentration could produce high flavonoid levels by altering the accumulation of secondary metabolites through the regulation of gene expression during synthesis. This regulation occurs by modulating gene transcription factors that play a major role in plant development processes, including the synthesis of secondary metabolites (Habibah et al., 2016). Tan et al. (2010) stated that most of the secondary metabolites were produced when culture entered the stationary phase. However, TFC produced in this study was obtained during the exponential phase of callus growth.

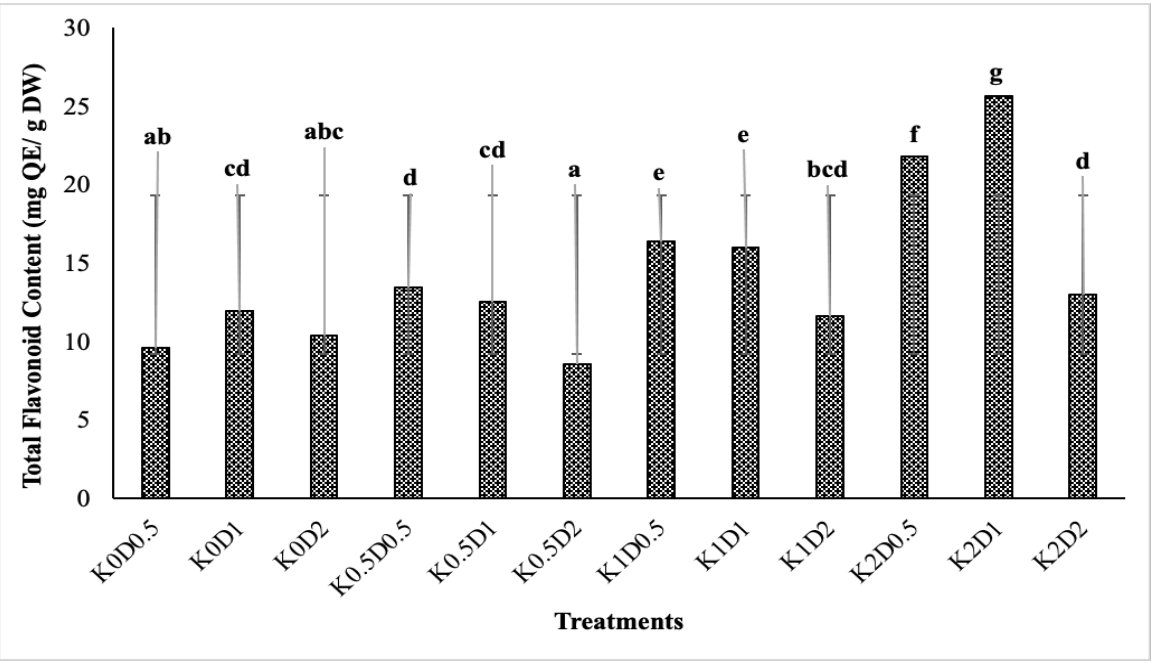

Figure 2. Effect of 2,4-D and kinetin concentration on total flavonoid content (TFC) of *T.paniculatum* callus at 28 days of culture

*) The bar shows standard deviation of mean (n=5). Different letters showing significant differences ($P \leq 0.05$)

CONCLUSION

In conclusion, this study showed that the study successfully determined the optimal concentration of 2,4-D and kinetin to increase callus biomass with flavonoid production from *T. paniculatum* callus culture. The results showed that the fastest callus formation was obtained 4 DAI, with callus texture varying from friable to intermediate, and compact. All treatments produced white, yellowish green, yellowish-white, brownish yellow, and pale green color of callus. The highest callus biomass of 0.105 g was produced from media with 2 mg/L 2,4-D and 1 mg/L kinetin. Meanwhile, the highest total flavonoid content of 25.66 mg QE/g DW was produced from media supplemented with 1 mg/L 2,4-D and 2 mg/L kinetin. This showed that callus biomass and flavonoid production required different concentrations of PGRs. Consequently, future studies were recommended to identify the type of flavonoid contained in *T. paniculatum* callus using HPLC and determine the antioxidant activity produced from in vitro culture.

AUTHORS' CONTRIBUTION

S.M.P.K. conducted the study and analyzed the data, R.R. designed and supervised all processes, wrote and reviewed the manuscript, while D.A. wrote and reviewed the manuscript.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest in this study.

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