INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
25–71/2025/34:340509
http://www.fspublishers.org

Full Length Article

Chitosan-induced Enhancement of Phenolic Compounds and Antioxidant Activity in *Dendrobium fimbriatum In Vitro* Cultures

Pruchayakorn Pawatung[1], Awipawadee Srisuk[2], Suvapat Khunthongchan[2], Maiporn Maipoka[3] and Sompop Saeheng[1,4,5*]

[1]*Division of Health and Applied Sciences (Biochemistry), Faculty of Science, Prince of Songkla University, 90110, Thailand*
[2]*Division of Biological Science (Biology), Faculty of Science, Prince of Songkla University, 90110, Thailand*
[3]*Rice Science Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, 73140, Thailand*
[4]*Center of Excellence for Biochemistry, Faculty of Science, Faculty of Science, Prince of Songkla University, Songkhla, Thailand*
[5]*Plant Cell and Physiology for Sustainable Agriculture Research Unit, Faculty of Science, Prince of Songkla Universitya, 90110, Thailand*
For correspondence: sompop.s@psu.ac.th; ORCID# 0000-0002-2455-7917; Telephone number +66-621047793
Received 22 January 2025; Accepted 11 July 2025; Published online 14 August 2025

Editor: Noreen Zahra

Abstract

Studies on the therapeutic potential of secondary metabolites derived from *Dendrobium* species have gained increasing attention in recent years, particularly due to their antioxidant, anticancer and anti-inflammatory properties. The inherently low production of secondary metabolites in *Dendrobium* species, combined with their slow growth in the wild, poses significant challenges in obtaining sufficient plant tissue for the extraction of bioactive compounds. Based on the hypothesis that supplementing plant tissue cultures with exogenous elicitors promotes biomass accumulation and enhances secondary metabolite production, this study investigated whether the biological elicitors chitosan and salicylic acid could increase total phenolic content and antioxidant activity in *Dendrobium fimbriatum*. *In vitro* shoot cultures of *D. fimbriatum* were treated with various concentrations of chitosan or salicylic acid for different durations. Colorimetric assays were used to assess the total phenolic content and antioxidant activity. The results showed that treating shoot tissue cultures with 100 mg/L chitosan for 30 days increased the total phenolic content from 14.73 ± 1.47 to 21.07 ± 1.01 mg GAE/g dry weight, a 30% increase. Moreover, this treatment did not affect photosynthetic pigments, suggesting there was no physiological stress. These results highlight the potential of chitosan as an effective elicitor for improving secondary metabolite production in *D. fimbriatum*. Future studies could focus on optimizing these conditions to further advance the production of bioactive compounds for use in herbal remedies derived from *D. fimbriatum*.

Keywords: *Dendrobium fimbriatum*; Chitosan; Salicylic acid; Antioxidant property; Secondary metabolites

Introduction

The epiphytic orchid *Dendrobium fimbriatum* is widely recognized as an ornamental plant species. In addition to its aesthetic value, several pharmacological activities have been documented in *Dendrobium* species, including anti-platelet aggregation (He *et al*. 2020) hepatoprotective effect (Wu *et al*. 2011) and anti-diabetic properties (Zhang *et al*. 2020). Given its rich reservoir of bioactive compounds with therapeutic properties, *D. fimbriatum* has been used in traditional medicine for decades (Wang *et al*. 2021). Numerous pharmacologically significant bioactive compounds have been identified in extracts from *D. fimbriatum*, including ayapin, β-sitosterol, and sesquiterpenes (Bi *et al*. 2003; Paul *et al*. 2017; Favre-Godal *et al*. 2022). Furthermore, alkaloids from *D. fimbriatum* have demonstrated various pharmacological effects; for instance, anosmines exhibit anti-inflammatory activity (Chen *et al*. 2018), while dendrobines have shown potential anti-tumor activity (Song *et al*. 2019). However, the extraction and purification of individual bioactive compounds from *D. fimbriatum* require substantial amounts of plant tissue. Like most orchids, *D. fimbriatum* grows relatively slowly in nature and necessitates specific growth conditions (Gurung and Gurung 2016). To address this limitation, tissue culture techniques have been employed to

To cite this paper: Pawatung P, A Srisuk, S Khunthongchan, M Maipoka, S Saeheng (2025). Chitosan-induced enhancement of phenolic compounds and antioxidant activity in *Dendrobium fimbriatum in vitro* cultures. *Intl J Agric Biol* 34:340509. https://doi.org/10.17957/IJAB/15.2394

accelerate the growth rate of *D. fimbriatum* under controlled conditions, providing a sustainable approach to increase biomass for pharmacological applications.

To enhance the production of secondary metabolites, various elicitors, including chitosan (Ferri and Tassoni 2011) and salicylic acid (Dong *et al*. 2010), have been applied to plant cell cultures. Both compounds are associated with plant responses to external inducers. Chitosan, a deacetylated derivative of chitin obtained from the shells of arthropods such as crabs and prawns, is known to trigger plant defense mechanisms. This activation leads to the release of secondary metabolites that help deter harmful insects. As a result, chitosan is widely used as an elicitor to enhance secondary metabolite production in plants (Ferri and Tassoni 2011). Molecular studies have demonstrated that chitosan interacts with transcription factors in the signal transduction pathways involved in secondary metabolite biosynthesis (Singh *et al.* 2020). Numerous studies have verified chitosan's effectiveness as a secondary metabolite enhancer. For instance, research on the medicinal plant *Momordica charantia* revealed that 10, 50 and 100 mg/L of chitosan significantly induced the production of phenolics, flavonoids, and anthocyanins in four-leaf stage seedlings (Sharifi-Rad *et al.* 2020). In *Dysphania ambrosioides* tissue cultures, secondary metabolite Z-ascaridole production was enhanced by applying 50–100 mg/L of chitosan (Carvalho *et al.* 2020).

Similarly, the medicinal orchid, *Coelogyne ovalis*, showed significant increases in total phenolic, flavonoid, and anthocyanin contents when 150 μM chitosan was applied to seedlings (Singh and Kumaria 2021). Furthermore, in another orchid species, *D. ovatum*, chitosan was identified as the most effective elicitor for inducing the production of moscatilin, a bioactive compound (Pujari *et al.* 2021). These results emphasize the potential of chitosan as an effective elicitor for inducing secondary metabolite production. However, to date, no study has investigated the effect of chitosan on the tissue culture of *D. fimbriatum*. In this study, *D. fimbriatum*, a medicinal orchid species, was used as a model plant to explore and validate the efficacy of chitosan in enhancing the biosynthesis of valuable secondary metabolites.

Salicylic acid (SA) is a naturally occurring organic acid derived from phenolic compounds. It functions as a plant hormone, playing a critical role in recognizing pathogen invasions and acting as an external growth regulator in physiological processes such as photosynthesis, growth, and root development (Miura and Tada 2014). Furthermore, SA stimulates the expression of defense-related genes and promotes the biosynthesis of secondary metabolites (Ali 2021; Rai *et al*. 2021). SA is also known to protect plants from various environmental stresses, including drought, high temperatures, salinity, and pathogen attacks. SA has been extensively utilized to stimulate the production of flavonoids and polyphenols in callus and tissue cultures of several plant species (Ali 2021). Moreover, SA has been reported to function as a non-living elicitor, enhancing the production of secondary metabolites such as terpenes, alkaloids, flavonoids, phytoalexins and essential oils (Silva *et al.* 2014; Gorni and Pacheco 2016). Research has shown that the use of SA plays a significant role in the synthesis of secondary metabolites in many plants. For example, SA increases polysaccharide production in *D. officinale* (Yuan *et al.* 2014) and enhances total flavonoid and phenolic content in *Crocus sativus* L. (Tajik *et al.* 2019). However, there is limited research on the use of SA treatment in tissue-cultured *D. fimbriatum* to increase the secondary metabolite production. This study aimed to enhance the total phenolic content and antioxidant activity in the *in vitro* shoot tissue cultures of *D. fimbriatum* by employing chitosan and salicylic acid as biological elicitors.

Material and Methods

Propagation of *D. fimbriatum* tissue culture shoots

D. fimbriatum plants were purchased from a local market and taxonomically identified. Newly emerging shoots were sterilized and propagated on a half-strength Murashige and Skoog (MS) medium with 7 mg/L of Phytoagar. The medium was supplemented with 0.5 mg/L 1-Naphthaleneacetic acid (NAA), 0.1 mg/L thidiazuron (TDZ) and 2 g/L peptone to support optimal growth. The pH of the medium was adjusted to 5.7 using 1 N potassium hydroxide (KOH). All cultures were maintained under controlled conditions, including illumination with cool daylight fluorescent lamps providing 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, a 16:8 light/dark cycle and a temperature of 25 ± 2°C.

Chitosan and salicylic acid treatment

One-month-old tissue-cultured shoots of *D. fimbriatum* were carefully transferred to fresh half-strength MS medium to assess the effects of elicitor treatments (Fig. 1). The experiment was designed to investigate the influence of two types of elicitors: low molecular weight chitosan and salicylic acid (SA). Shoots were placed in media either without any supplement, serving as the control or supplemented with varying concentrations of chitosan (5, 10, 20, 50 and 100 mg/L) or salicylic acid (25, 50, 75, 100 and 150 μM).

To examine the time-dependent effects of these treatments, the cultures were maintained for three different durations—7, 15 and 30 days—under controlled environmental conditions. The tissue cultures were incubated in a growth chamber with a 16-hour light and 8-hour dark photoperiod, illuminated by cool white fluorescent lamps providing a light intensity of approximately 40 $\mu mol.m^{-2}.s^{-1}$. The temperature was maintained at 25 ± 2°C with relative humidity ranging from 60 to 70%.

Fig. 1: One-month-old *in vitro* shoot culture of *D. fimbriatum* in half-strength MS medium

At each time point, the shoots were harvested, and their fresh weight was recorded. The samples were then immediately frozen in liquid nitrogen to preserve their metabolic state. Subsequently, the frozen tissues were ground to a fine powder, lyophilized to remove moisture, and stored at –80°C until further biochemical analyses. Each treatment and time-point combination included at least three biological replicates to ensure statistical reliability.

Secondary metabolite extraction

The 50 mg of ground shoot tissue was extracted in 5 mL of ice-cold 70% ethanol with regular shaking for 2 h at 4°C. After centrifugation at 13,000 rpm for 5 min, the clear supernatant was collected. The clear supernatant was divided into 4 portions which were used to determine (1) total phenolic content, (2) antioxidant capacity *via* 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay, (3) antioxidant capacity *via* 2,2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and (4) photosynthetic pigment contents.

Total phenolic contents

Each reaction was prepared by mixing 100 μL of the extract with 200 μL of 10% Folin-Ciocalteu reagent, followed by vortexing and allowing the mixture to react for 5 min before adding 800 μL of 700 m*M* sodium carbonate (Na_2CO_3). A blank (70% ethanol) and a standard solution of gallic acid were included. The reaction was incubated at room temperature in the dark for 1 h and then was centrifuged at 13,000 rpm for 5 min to remove any precipitates. Finally, 200 μL of the clear supernatant was transferred to a 96-well plate, and absorbance was measured at 765 nm using a microplate reader (Ainsworth and Gillespie 2007). Total phenolic content was calculated based on a gallic acid calibration curve.

Antioxidant capacity *via* DPPH and ABTS

To determine antioxidant capacity, the DPPH assay was performed with modifications based on a previous study (Sirisarn *et al.* 2024). The DPPH solution was prepared by dissolving DPPH in absolute methanol until the solution reached an OD_{517} of 0.8. In a 96-well plate, each reaction included 190 μL of working DPPH solution and 20 μL of extracts, blank or standard. The reactions were incubated in the dark at room temperature for 30 min before absorbance was measured at OD_{517}. For the ABTS assay, the working ABTS solution was prepared by mixing 7 m*M* ABTS and 2.45 m*M* potassium persulfate ($K_2S_2O_8$), which was kept in the dark for 16 h before dilution to an OD_{734} of 0.8 (Sirisarn *et al.* 2024). In the 96-well plate assay, 180 μL of the working ABTS solution and 20 μL of extracts, blank or standard were added, followed by measurement at OD_{734}. Antioxidant capacity for both assays was calculated based on the Trolox calibration curve, expressed as mg Trolox equivalent per g of dry weight.

Total chlorophyll and carotenoid contents

The content of photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids (Chl *a*, Chl *b*, total chlorophyll and carotenoids), was determined following the previous methods (Batool *et al.* 2020; Shemi *et al.* 2021). A 50 μL aliquot of the supernatant from each extracted sample was used to measure absorbance at 470, 645 and 663 nm. The chlorophyll and carotenoid content were calculated using the following formulas, V represented the volume of the extract (cm^3) and W was recorded from fresh weight (mg).

$$\text{Chlorophyll } a \text{ content} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times V / (1000 \times W) \quad (1)$$

$$\text{Chlorophyll } b \text{ content} = (22.7 \times A_{645} - 4.68 \times A_{663}) \times V / (1000 \times W) \quad (2)$$

$$\text{Total chlorophyll content} = (20.2 \times A_{645} + 8.02 \times A_{663}) \times V / (1000 \times W) \quad (3)$$

$$\text{Carotenoids} = (1000 \times A_{470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b) / 229 \quad (4)$$

Statistical analysis

Each treatment was conducted in at least three biological replicates unless otherwise stated. Statistical analyses were

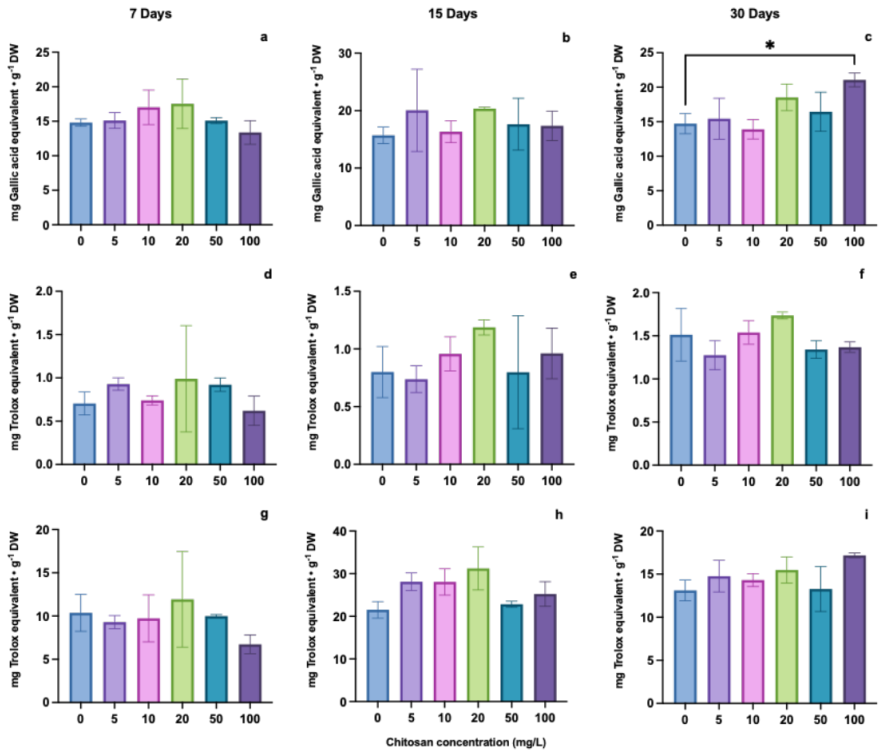

Fig. 2: The effect of chitosan treatments on total phenolics and antioxidant capacity of *in vitro* shoot culture of *D. fimbriatum* (**a-i**). Total phenolic contents (**a-c**): 7-day treatment (**a**), 15-day treatment (**b**) and 30-day treatment (**c**). Antioxidant capacity *via* DPPH assay (**d-f**): 7-day treatment (**d**), 15-day treatment (**e**) and 30-day treatment (**f**). Antioxidant capacity *via* ABTS assay (**g-i**): 7-day treatment (**g**), 15-day treatment (**h**) and 30-day treatment (**i**)

performed using GraphPad software. Data were tested for normality using the Shapiro-Wilk test and for homogeneous variance using the Bartlett test. Analysis of variance (ANOVA) followed by Dunnett's test was used for comparison analysis, with significant differences considered at $P < 0.05$.

Results

Effects of chitosan on total phenolic contents and antioxidant capacity in the whole shoot of *D. fimbriatum*

To investigate the impact of chitosan as a biological elicitor on the induction of secondary metabolites in *D. fimbriatum*, 1-month-old *in vitro* shoot cultures were treated with varying concentrations of chitosan (0, 5, 10, 20, 50 and 100 mg/L) and maintained for 7, 15 and 30 days (Fig. 2). Phenolics, a major group of secondary metabolites, were quantified and compared to the control group (untreated with chitosan). The results showed that treatment with 100 mg/L of chitosan for 30 days significantly enhanced phenolic production in the shoot tissue of *D. fimbriatum* ($P < 0.05$) (Fig. 2c). Specifically, the total phenolic content increased from 14.73 ± 1.47 mg gallic acid equivalents per gram dry weight (mg GAE/g DW) in the control to 21.07 ± 1.01 mg GAE/g DW under the 100 mg/L chitosan treatment, representing an approximate 30% increase, demonstrating a potential approach for inducing phenolic production in *D. fimbriatum* through the *in vitro* shoot culture method. Phenolic compounds are known to contribute to antioxidant capacity, which is defined as the ability of a compound to donate electrons and neutralize free radicals. Therefore, we further evaluated the antioxidant capacity of *D. fimbriatum* in response to chitosan treatment using two assays: DPPH and ABTS. However, no significant increase in antioxidant capacity *via* DPPH (Fig. 2d-f) and ABTS (Fig. 2g-i) assays was observed for any chitosan treatment compared to the control condition.

Effects of salicylic acid on total phenolic contents and antioxidant capacity in the whole shoot of *D. fimbriatum*

Salicylic acid is another well-known biological elicitor often used to enhance secondary metabolite production. In this study, various concentrations of salicylic acid (25, 50, 75, 100 and 150 μM) were introduced to 1-month-old *in vitro* shoot cultures of *D. fimbriatum* for three specific durations: 7 days, 15 days and 30 days. As phenolics are the primary compounds in secondary metabolites, total phenolic content was quantified, as shown in (Fig. 4a, b, c). However, no significant increase in total phenolic content was observed in any salicylic acid treatments compared to the control condition.

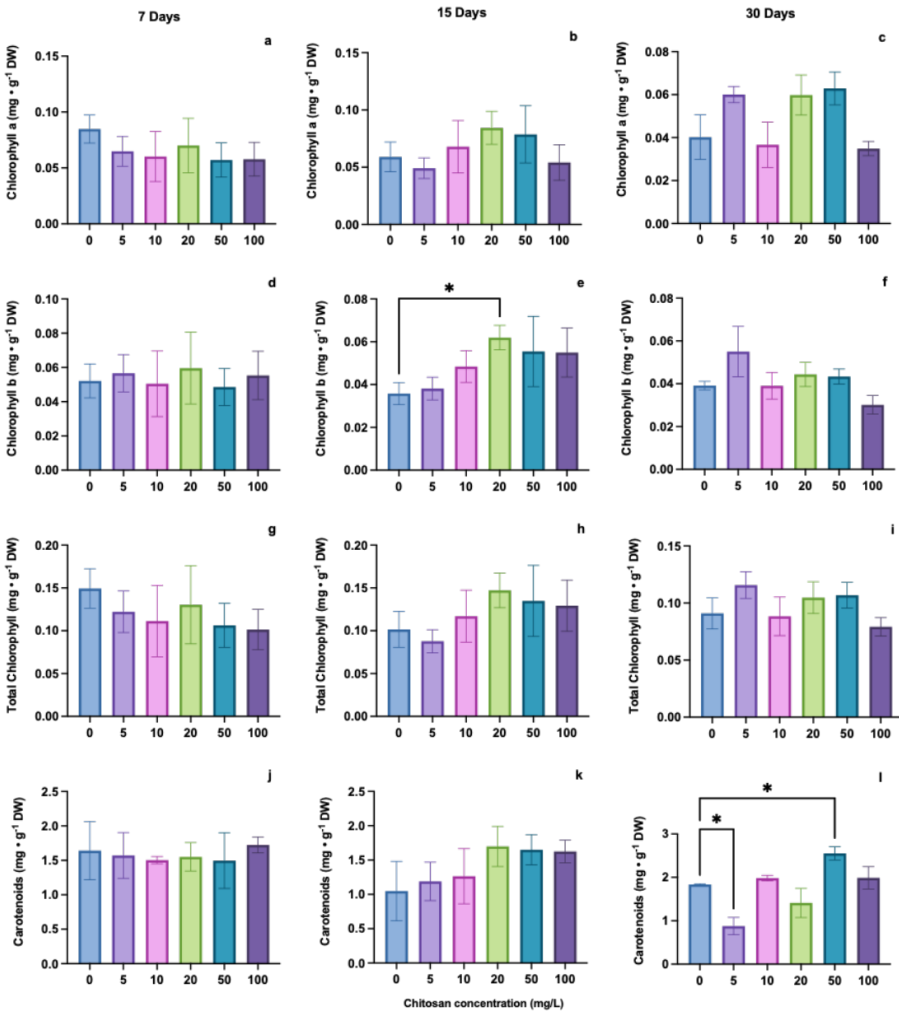

Fig. 3: The effect of Chitosan treatments on photosynthetic pigment contents of *in vitro* shoot culture of *D. fimbriatum* (**a-l**). Chlorophyll *a* contents (**a-c**): 7-day treatment (**a**), 15-day treatment (**b**) and 30-day treatment (**c**). Chlorophyll *b* contents (**d-f**): 7-day treatment (**d**), 15-day treatment (**e**) and 30-day treatment (**f**). Total Chlorophyll contents (**g-i**): 7-day treatment (**g**), 15-day treatment (**h**) and 30-day treatment (**i**). Carotenoid contents: 7-day treatment (**j**), 15-day treatment (**k**) and 30-day treatment (**l**)

To further evaluate the effects of salicylic acid, the antioxidant capacity of the shoot tissues was assessed using DPPH and ABTS assays. Similar to the results for total phenolic content, no significant increase in antioxidant capacity *via* DPPH (Fig. 4d-f) and ABTS (Fig. 4g-i) was detected for any treatment. These findings suggest that salicylic acid is not a suitable biological elicitor for enhancing the production of secondary metabolites or improving antioxidant properties in the *in vitro* shoot cultures of *D. fimbriatum*.

Effects of chitosan on photosynthetic pigment contents in the whole shoot of *D. fimbriatum*

The results indicated that chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were not significantly affected (Fig. 3a-d) by any concentration of chitosan treatment, except for an increase in chlorophyll *b* observed with 20 mg/L chitosan after 15 days of treatment, rising from 0.035 ± 0.01 to 0.06 ± 0.01 mg/g DW ($P < 0.05$) (Fig. 3e).

Similarly, total carotenoid content showed no significant differences across chitosan concentrations, except after 30 days of treatment, where contrasting trends were observed (Fig. 3l). A significant decrease in carotenoid content was recorded at 5 mg/L chitosan, from 1.84 ± 0.01 to 0.88 ± 0.20 mg/g DW ($P < 0.05$), while a significant increase was observed at 50 mg/L chitosan, from 1.84 ± 0.01 to 2.55 ± 0.15 mg/g DW ($P < 0.05$).

Overall, these findings suggest that a 30-day treatment with 100 mg/L chitosan is the optimal condition for inducing secondary metabolite production in the *in vitro* shoot cultures of *D. fimbriatum*. Furthermore, this condition does not negatively impact total photosynthetic pigment levels, indicating that the plants are not under stress.

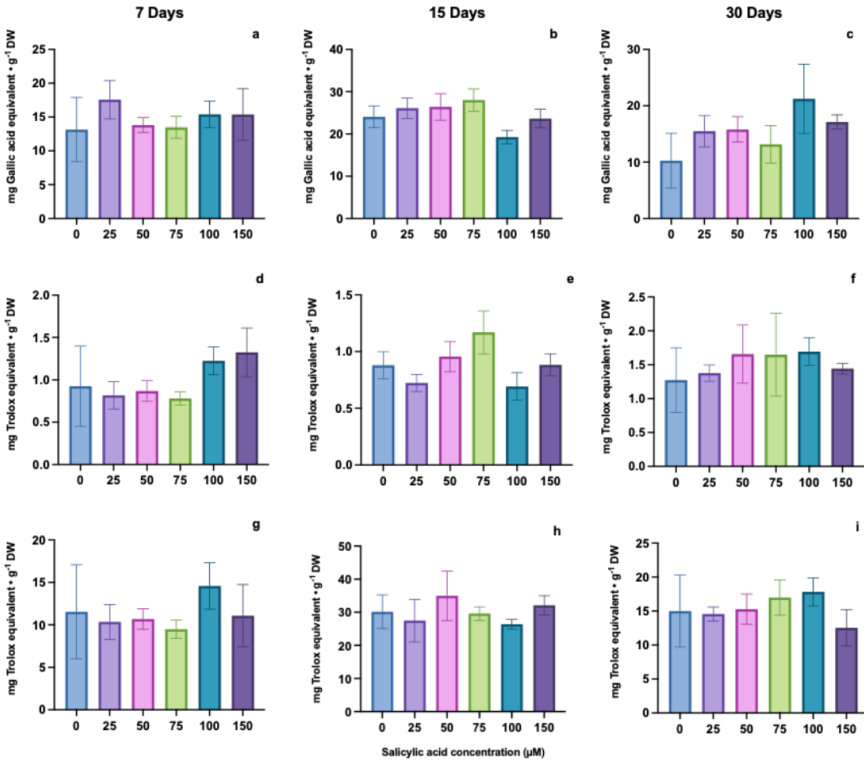

Fig. 4: The effect of salicylic acid treatments on total phenolics and antioxidant capacity of *in vitro* shoot culture of *D. fimbriatum* (**a-i**). Total phenolic contents (**a-c**): 7-day treatment (**a**), 15-day treatment (**b**) and 30-day treatment (**c**). Antioxidant capacity *via* DPPH assay (**d-f**): 7-day treatment (**d**), 15-day treatment (**e**) and 30-day treatment (**f**). Antioxidant capacity *via* ABTS assay (**g-i**): 7-day treatment (**g**), 15-day treatment (**h**) and 30-day treatment (**i**)

Effects of salicylic acid on photosynthetic pigment contents in the whole shoot of *D. fimbriatum*

In this study, all photosynthetic pigment contents were measured. However, no significant increase in chlorophyll *a* (Fig. 5a-c), chlorophyll *b* (Fig. 5d-f) and total chlorophyll (Fig. 5g-i) was observed across various salicylic acid concentrations and treatment durations (7-day, 15-day and 30-day treatments). Additionally, carotenoid content was analyzed to assess whether the plants experienced stress. Similarly, no significant decrease in carotenoid content (Fig. 5j-l) was detected in any salicylic acid treatment compared to the control (without salicylic acid supplementation). These results collectively suggest that salicylic acid does not exert a detectable impact on photosynthetic pigments under the controlled conditions of this study.

Discussion

Dendrobium species are among the key orchids studied for their medicinal and pharmaceutical properties (Li *et al.* 2023a). Furthermore, tissue culture techniques represent an effective approach for propagating plants with a slow-growing nature, such as orchids. Previous studies have demonstrated that various biological elicitors can serve as tools to induce secondary metabolites, which are essential chemical compounds with pharmaceutical significance. For instance, research on the medicinal orchid *Coelogyne ovalis* revealed that treatments with chitosan, methyl jasmonate, and salicylic acid induced the accumulation of flavonoids and total phenolic content in specific organs (Singh and Kumaria 2021). Similarly, chitosan, methyl jasmonate and salicylic acid were shown to enhance the accumulation of moscatilin, a bioactive compound, in tissue cultures of *Dendrobium ovatum* (Pujari *et al.* 2021). However, it is noteworthy that elicitors may stimulate the production of secondary metabolites in certain plants but not in others. For example, in tissue cultures of *Dendrobium*, salicylic acid, but not methyl jasmonate, was reported to induce anthocyanin accumulation (Malik *et al.* 2021). This variability underscores the importance of investigating the specific elicitors and their optimal concentrations required to trigger secondary metabolite production in different plant species. In the present study, we demonstrated for the first time that chitosan at a concentration of 100 mg/L successfully induced the accumulation of phenolic compounds in *D. fimbriatum*.

Phenolic compounds are primarily synthesized through the phenylpropanoid biosynthetic pathway (Rosa *et al.* 2019). A previous study demonstrated that the optimal concentration of chitosan activated the expression of genes involved in this pathway in *Haplophyllum virgatum* cell

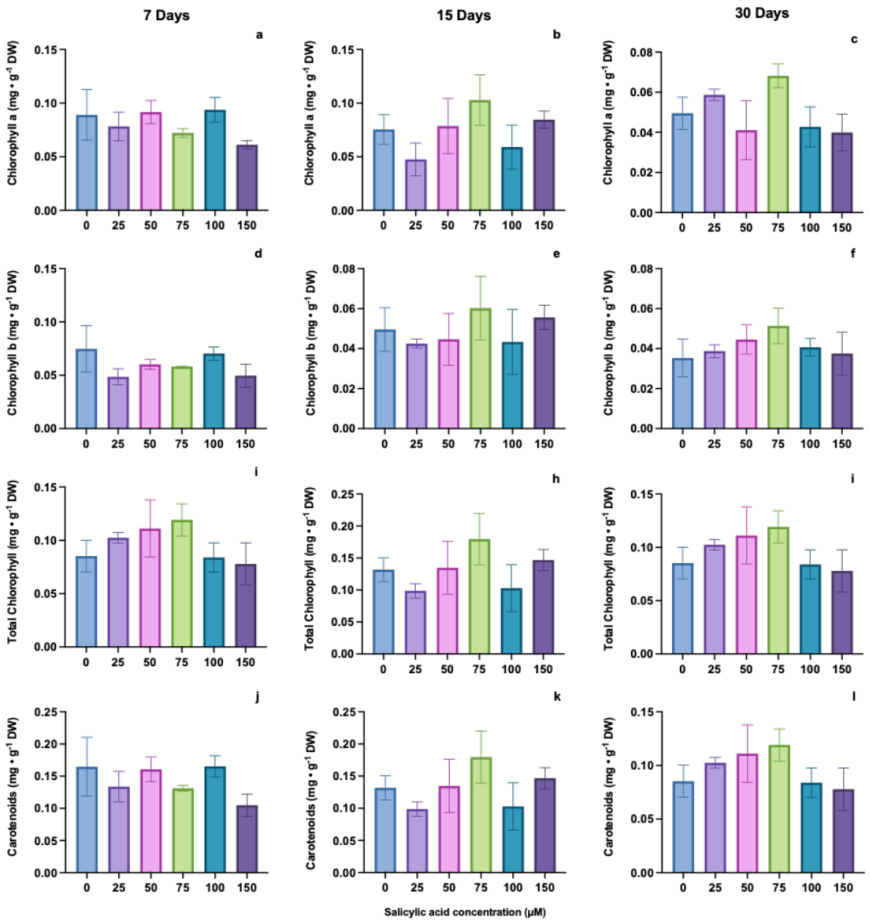

Fig. 5: The effect of salicylic acid treatments on photosynthetic pigment contents of *in vitro* shoot culture of *D. fimbriatum* (**a-l**). Chlorophyll *a* contents (**a-c**): 7-day treatment (**a**), 15-day treatment (**b**) and 30-day treatment (**c**). Chlorophyll *b* contents (**d-f**): 7-day treatment (**d**), 15-day treatment (**e**) and 30-day treatment (**f**). Total Chlorophyll contents (**g-i**): 7-day treatment (**g**), 15-day treatment (**h**) and 30-day treatment (**i**). Carotenoid contents: 7-day treatment (**j**), 15-day treatment (**k**) and 30-day treatment (**l**)

suspension cultures (Torabi *et al.* 2023). Based on this, it is reasonable to anticipate that the optimal concentration of chitosan used in our study may similarly induce the expression of genes related to phenylpropanoid biosynthesis, thereby enhancing phenolic production in *D. fimbriatum*. However, our investigation was limited to the measurement of total phenolics and antioxidant activity using colorimetric assays. We did not examine the underlying cellular mechanisms or specific metabolic changes that might contribute to increased secondary metabolite production, particularly phenolic compounds.

Contrary to expectations, salicylic acid treatments across all concentrations and durations did not significantly enhance phenolic content or antioxidant activity in *D. fimbriatum* in our study. These results diverge from studies in other medicinal plants, such as *Prunella vulgaris* and *Dendrobium officinale*, where SA was shown to promote secondary metabolite accumulation and antioxidant activity (Feng *et al.* 2021; Tang *et al.* 2023). The lack of response in *D. fimbriatum* may be attributed to species-specific metabolic pathways. Interestingly, SA has been shown to enhance flavonoid and anthocyanin content in certain orchid species, such as *Phalaenopsis pulcherrima* and *Dendrobium ovatum*, but at specific concentrations and treatment durations (Smetanska 2008; Pujari *et al.* 2021; Chashmi *et al.* 2023). These findings highlight the importance of optimizing SA concentrations and exposure times for each plant species to maximize its elicitation potential.

Despite the increased phenolic content, no corresponding improvement in antioxidant capacity, assessed *via* DPPH and ABTS assays, was observed. This suggests that the phenolic compounds induced by chitosan may not significantly contribute to radical scavenging activity or the induced phenolic profile may differ from compounds with strong antioxidant potential, similar to findings from a previous study on antioxidant activity in 32 selected herbs (Wojdyło *et al.* 2007). Similar discrepancies between phenolic accumulation and antioxidant activity have been observed in *Linum album* and *Coelogyne ovalis*, where phenolic compounds with varied bioactivities were differentially induced by chitosan and other elicitors (Samari *et al.* 2020; Singh and Kumaria 2021).

Both chlorophyll and carotenoid levels change dynamically with plant age or in response to stress (Munné-Bosch and Alegre 2002; Huang *et al.* 2016; Li *et al.* 2023b). Therefore, the activation of elicitors may simultaneously induce stress in plants. In our study, chitosan-treated shoots maintained stable levels of photosynthetic pigments, with slight increases in chlorophyll *b* and carotenoid content at specific concentrations, along with enhanced accumulation of phenolics stimulated by chitosan. This indicates that no significant stress was imposed on plant growth during the treatment. These findings are consistent with studies in other *Dendrobium* species, where elicitors such as chitosan supported plant growth and stress resilience without significantly affecting photosynthesis (Malik *et al.* 2021). Conversely, SA treatments had no measurable impact on pigment levels, corroborating reports that high SA concentrations or specific treatment conditions may not enhance photosynthesis (Chashmi *et al.* 2023).

Conclusion

A suitable elicitor can be employed to induce secondary metabolite production in medicinal orchids. In this study, we identified chitosan as a promising elicitor for enhancing phenolic production in *D. fimbriatum* without compromising photosynthetic pigment levels, highlighting its potential utility in tissue culture-based metabolite production systems. Future research should focus on characterizing the specific phenolic compounds induced by chitosan, optimizing elicitor concentrations, and elucidating the molecular mechanisms underlying the elicitation process. Furthermore, combining chitosan with other elicitors, such as methyl jasmonate, may synergistically enhance metabolite production, as observed in other medicinal plants. Advances in pharmaceutical research and tissue culture techniques could further accelerate the discovery of novel drugs.

Acknowledgments

This research was financially supported by the Faculty of Science Research Fund (Contract No. SCIGEN66002). The authors would like to thank Associate Professor Dr. Upatham Meesawat for providing guidance on tissue culture techniques and supplying the shoot tissue cultures of *Dendrobium fimbriatum*.

Author Contributions

SS was responsible for research design, investigation, data analysis, data interpretation, manuscript preparation, and manuscript revision. PP contributed to data analysis, data interpretation, manuscript preparation, and manuscript revision. AS and SK were responsible for conducting experiments, data analysis, and data interpretation. MM contributed to manuscript preparation and manuscript revision. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest associated with this study.

Data Availability

All necessary data have been included in the manuscript.

Ethics Approval

Ethical approvals were unnecessary for the conduct of this research.

Reference

Ainsworth EA, KM Gillespie (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using folin–ciocalteu reagent. *Nat Protoc* 2:875–877

Ali B (2021). Salicylic acid: An efficient elicitor of secondary metabolite production in plants. *Biocatal Agric Biotechnol* 31:101884

Batool T, S Ali, MF Seleiman, NH Naveed, A Ali, K Ahmed, M Abid, M Rizwan, MR Shahid, M Alotaibi, I Al-Ashkar, M Mubashar (2020). Plant growth promoting rhizobacteria alleviates drought stress in potato in response to suppressive oxidative stress and antioxidant enzymes activities. *Sci Rep* 10:1-19

Bi Z, Z Wang, L Xu, G Xu (2003). Studies on the chemical constituents of *Dendrobium fimbriatum*. *Acta Pharm Sin* 38:526–529

Carvalho AAD, SKV Bertolucci, ADC Honorato, TT Rocha, ST Silva, JEBP Pinto (2020). Influence of light spectra and elicitors on growth and ascaridole content using *in vitro* cultures of *Dysphania ambrosioides* L. *Plant Cell Tissue Org Cult* 143:277–290

Chashmi KA, VOG Omran, R Ebrahimi, H Moradi, V Abdosi (2023). *In-vitro* elicitation of *Phalaenopsis pulcherrima* leaf explants using melatonin, salicylic acid and methyl jasmonate for plbs induction and anthocyanin production. *Biol Bull* 50:379–389

Chen H, X Li, Y Xu, K Lo, H Zheng, H Hu, J Wang, Y Lin (2018). Study on the polar extracts of *Dendrobium nobile*, *D. officinale*, *D. loddigesii*, and *Flickingeria fimbriata*: Metabolite identification, content evaluation, and bioactivity assay. *Molecules* 23:1-10

Dong J, G Wan, Z Liang (2010). Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. *J Biotechnol* 148:99–104

Favre-Godal Q, J Hubert, A Kotland, D Garnier, C Beaugendre, L Gourguillon, A Urbain, S Lordel-Madeleine, P Choisy (2022). Extensive phytochemical assessment of *Dendrobium fimbriatum* hook (orchidaceae). *Nat Prod Commun* 17:1–7

Feng X, D Cui, L Zeng, Z Wu, X Xie, J Zhang, J Huang, X Zhang (2021). Effects of UV-B irradiation alone and in combination with salicylic acid on the growth and active ingredients of *Dendrobium officinale*. *Russ J Plant Physiol* 68:483–490

Ferri M, A Tassoni (2011). Chitosan as elicitor of health beneficial secondary metabolites in *in vitro* plant cell cultures. *In: Handbook of Chitosan Research and Applications*, pp:389–414. Mackay RG, JM Tait (Eds.). Nova Science Publishers, New York

Gorni PH, AC Pacheco (2016). Growth promotion and elicitor activity of salicylic acid in *Achillea millefolium* L. *Afr J Biotechnol* 15:657–665

Gurung A, C Gurung (2016). Domestication and *ex situ* conservation of three species of *Dendrobium* Swartz (Orchidaceae) under greenhouse conditions. *Pleione* 10:323–332

He L, Q Su, L Bai, M Li, J Liu, X Liu, C Zhang, Z Jiang, J He, J Shi, S Huang, L Guo (2020). Recent research progress on natural small molecule bibenzyls and its derivatives in *Dendrobium* species. *Euro J Med Chem* 204:112530

Huang C, D Wang, L Sun, L Wei (2016). Effects of exogenous salicylic acid on the physiological characteristics of *Dendrobium officinale* under chilling stress. *Plant Growth Regul* 79:199–208

Li PY, L Li, YZ Wang (2023a). Traditional uses, chemical compositions and pharmacological activities of *Dendrobium*: A review. *J Ethnopharmacol* 310:116382

Li X, Y Fan, J Ma, X Gao, G Wang, S Wu, Y Liu, K Yang, E Xu, S Pu, A Luo (2023b). Cerium improves the physiology and medicinal components of *Dendrobium nobile* Lindl. under copper stress. *J Plant Physiol* 280:153896

Malik ANA, J Uddain, CK Chin, BL Chew, S Subramaniam (2021). Elicitation of protocorm-like bodies (PLBs) of dendrobium 'sabin blue'using methyl jasmonate, salicylic acid and melatonin for *in vitro* production of anthocyanin. *Phytochem Lett* 43:60–64

Miura K, Y Tada (2014). Regulation of water, salinity, and cold stress responses by salicylic acid. *Front Plant Sci* 5:1-12

Munné-Bosch S, L Alegre (2002). Plant aging increases oxidative stress in chloroplasts. *Planta* 214:608–615

Paul P, M Joshi, D Gurjar, S Shailajan, S Kumaria (2017). *In vitro* organogenesis and estimation of *β*-sitosterol in *Dendrobium fimbriatum* hook.: An orchid of biopharmaceutical importance. *S Afr J Bot* 113:248–252

Pujari I, A Thomas, PS Rai, K Satyamoorthy, VS Babu (2021). *In vitro* bioproduction and enhancement of moscatilin from a threatened tropical epiphytic orchid, *Dendrobium ovatum* (willd.) kraenzl. *3 Biotech* 11:1-20

Rai KK, N Pandey, N Rai, SK Rai, S Pandey-Rai (2021). Salicylic acid and nitric oxide: insight into the transcriptional regulation of their metabolism and regulatory functions in plants. *Front Agron* 3:1-22

Rosa LADL, JO Moreno-Escamilla, J Rodrigo-García, E Alvarez-Parrilla (2019). Phenolic compounds. *Postharvest Physiology and Biochemistry of Fruits and Vegetables*, pp:253-271. Elsevier

Samari E, M Sharifi, F Ghanati, E Fuss, NA Chashmi (2020). Chitosan-induced phenolics production is mediated by nitrogenous regulatory molecules: No and pas in linum album hairy roots. *Plant Cell Tissue Org Cult* 140:563–576

Sharifi-Rad R, SE Bahabadi, A Samzadeh-Kermani, M Gholami (2020). The effect of non-biological elicitors on physiological and biochemical properties of medicinal plant *Momordica charantia* L. *Iran J Sci Technol Transact A Sci* 44:1315–1326

Shemi R, R Wang, ESM Gheith, HA Hussain, S Hussain, M Irfan, L Cholidah, K Zhang, S Zhang, L Wang (2021). Effects of salicylic acid, zinc and glycine betaine on morpho-physiological growth and yield of maize under drought stress. *Sci Rep* 11:1-14

Silva SD, CB Moreira, MA Esquibel, RS Gil, C Riehl, A Sato (2014). Effect of salicylic acid on essential oil compounds of *Melissa officinalis in vitro* plants. *Agropec Técn* 35:178–184

Singh N, S Kumaria (2021). Deciphering the role of stress elicitors on the differential modulation of chalcone synthase gene and subsequent production of secondary metabolites in micropropagated *Coelogyne ovalis* Lindl., a therapeutically important medicinal orchid. *S Afr J Bot* 140:336–348

Singh T, U Sharma, V Agrawal (2020). Isolation and optimization of plumbagin production in root callus of *Plumbago zeylanica* L. augmented with chitosan and yeast extract. *Industr Crops Prod* 151:1-14

Sirisarn AMW, K Kankamol, S Sompop (2024). Exploring *Bougainvillea glabra* flowers: A promising source of natural antimicrobial and anticancer agents. *J Appl Biol Biotechnol* 12:174–184

Smetanska I (2008). Production of secondary metabolites using plant cell cultures. *Food Biotechnol* 111:187–228

Song TH, XX Chen, CKF Lee, SCW Sze, YB Feng, ZJ Yang, HY Chen, ST Li, LY Zhang, G Wei (2019). Dendrobine targeting jnk stress signaling to sensitize chemotoxicity of cisplatin against non-small cell lung cancer cells *in vitro* and *in vivo*. *Phytomedicine* 53:18–27

Tajik S, F Zarinkamar, BM Soltani, M Nazari (2019). Induction of phenolic and flavonoid compounds in leaves of saffron (*Crocus sativus* L.) by salicylic acid. *Sci Hortic* 257:108751

Tang H, J Hu, M Zhao, L Cao, Y Chen (2023). Comparative study of the physiological responses, secondary metabolites, and gene expression of medicinal plant *Prunella vulgaris* L. treated with exogenous methyl jasmonate and salicylic acid. *Acta Physiol Plant* 45:20

Torabi S, F Karimi, K Razavi (2023). Phenylpropanoid biosynthetic gene expression in cell suspension culture of *Haplophyllum virgatum Spach.* under chitin treatment. *In Vitro Cell Dev Biol Plant* 59:49–60

Wang Z, W Jiang, Y Liu, X Meng, X Su, M Cao, L Wu, N Yu, S Xing, D Peng (2021). Putative genes in alkaloid biosynthesis identified in *Dendrobium officinale* by correlating the contents of major bioactive metabolites with genes expression between protocorm-like bodies and leaves. *BMC Genom* 22:1–17

Wojdyło A, J Oszmiański, R Czemerys (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem* 105:940–949

Wu LY, CW Cheng, YC Tien, CL Kuo, SF Lo, WH Peng (2011). Anti-oxidative and hepatoprotective activities of *Dendrobium tosaense* and *Ephemerantha fimbriata* in carbon tetrachloride-induced acute liver injury. *J Chin Med* 22:47–63

Yuan Z, G Cong, J Zhang (2014). Effects of exogenous salicylic acid on polysaccharides production of *Dendrobium officinale*. *S Afr J Bot* 95:78–84

Zhang Q, J Li, M Luo, GY Xie, W Zeng, Y Wu, Y Zhu, X Yang, AY Guo (2020). Systematic transcriptome and regulatory network analyses reveal the hypoglycemic mechanism of *Dendrobium fimbriatum*. *Mol Ther Nucl Acids* 19:1–14