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뚜껑덩굴 부정근 배양에 암조건 및 광조건 처리가 대사물 변화와 항산화 활성에 미치는 효과

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Impact of Dark and Light Treatment on Metabolic Changes and Antioxidant Activities in Adventitious Root Culture of *Actinostemma lobatum*

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ABSTRACT

Background: Tissue culture is an important strategy for metabolite accumulation in plants. Moreover, adventitious roots can produce high levels of phytochemicals. *Actinostemma lobatum* is consumed as a medicinal plant in South Korea, China, India, and Thailand. In this study, we aimed to identify the effects of darkness and light on metabolic changes and antioxidant activity in the adventitious roots of *A. lobatum*.

Methods and Results: To confirm metabolic changes and antioxidant activity, adventitious roots of *A. lobatum* were grown under appropriate conditions and separated into dark and light treatment groups. Light conditions were found to more significantly influence the accumulation of natural pigments, such as chlorophyll *a* [0.136 ± 0.001 $mg \cdot g^{-1}$ dry weight (DW)] and total carotenoids (0.047 ± 0.001 $mg \cdot g^{-1}$ DW), than dark conditions (0.072 ± 0.001 $mg \cdot g^{-1}$ DW and 0.026 ± 0.003 $mg \cdot g^{-1}$ DW, respectively). Moreover, the light treatment group had substantially high contents of total phenolic, flavonoid, and rutin compounds (11.273 ± 0.291 gallic acid equivalent (GAE)·$mg \cdot g^{-1}$ DW, 9.943 ± 0.28 quercetin equivalent (QE)·$mg \cdot g^{-1}$ DW, and 2.136 ± 0.491 $mg \cdot g^{-1}$ DW, respectively). These results revealed that light stimulates the accumulation of secondary metabolites, such as phenolics and flavonoids, and enhances antioxidant activity in the adventitious roots of *A. lobatum*.

Conclusions: Light conditions can considerably influence the production of health-beneficial metabolites in the adventitious roots of *A. lobatum.* This provides an understanding of metabolism in adventitious roots for further experimental studies on environmentally sustainable plant secondary metabolite production. In this study, we show that suitable root cultures have the potential to be used as supplements in the pharmaceutical and nutraceutical industries.

Key Words: *Actinostemma lobatum*, Adventitious Root, Exposure of Dark and Light, Phenolics, Antioxidant Activities

INTRODUCTION

Actinostemma lobatum is a herbaceous annual plant in Asia, such as South Korea, China, India, and Thailand (Zheng *et al*., 2020). Historically, the whole plant of *A. lobatum* was considered a folklore medicinal plant, consumed as a diuretic and for the

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treatment of diverse chronic diseases (Kim *et al*., 2008; Cao *et al*., 2015).

Specifically, several earlier studies reported the anti-oxidative (Kim, 2010), anti-thrombotic (Kim *et al*., 2008), anti-tumor (Fujioka *et al*., 1996; Li *et al*., 2012), antifungal (Choi *et al*., 2024), and antimicrobial (Chen *et al*., 2005) activity in *A. lobatum* extracts, suggesting potential therapeutic applications. Lee *et al*. (2024) reported that the *A. lobatum* contained a high level of flavonoids, such as quercetin and kaempferol, which closely correlate with antibiofilm and antihemolytic activities; moreover, Cao *et al*. (2015) isolated triterpene saponins from *A. lobatum*, supporting the inhibition activity of the cytotoxicity. Underlying this knowledge, in the present study, we explored the effects of light and dark on adventitious roots of *A. lobatum*.

In vitro tissue cultures are being developed as a potent strategy for producing biologically useful phytochemicals (Hussain *et al*., 2022). Especially, the adventitious roots-inducing strategy could enhance the production of pharmaceutically important metabolites (Khanam *et al*., 2024), following rapid biomass production under sterile conditions (Murthy *et al*., 2024) without gene modifications.

Additionally, previous studies reported that depending on the incubation conditions, the accumulation of phytochemicals considerably impacted plant roots (Park *et al*., 2022; Yun *et al*., 2022). In other words, due to the convenient strategy and high yield of biomass, adventitious root culture, such as eco-friendly and sustainable production of phytochemicals, provided a convenient strategy and high yield of biomass, highlighting the accumulation of secondary metabolites in recent studies.

For the generation and development of plants, environmental factors are essential, including temperature, salt, soil, light, water, pH, and oxygen; moreover, they have significant effects on the production of secondary metabolites (Park *et al*., 2024). Among them, light is the crucial factor for plant development, controlling the production of plant metabolites (Kapoor *et al*., 2018).

Several previous studies revealed that light radiation, direction, and intensity cause different accumulations of metabolites in plants (Yang *et al*., 2018). According to Bungala *et al*. (2024), different exposed LEDs significantly influenced the biomass, secondary metabolites, and antioxidant activities in *Brassica rapa subsp. chinensis*, and Park *et al*. (2024) reported that the light irradiation considerably impacted the production of primary and secondary metabolites in *Althaea officinalis* hairy root.

In plants, phenolic compounds are a large group of secondary metabolites that are essential in metabolism (Dwivedi *et al*., 2016) and have been demonstrated to have various activities, such as development, signaling, organogenesis, UV protection, pathogen defense, and response to biotic stress. (Bauters *et al*., 2021; Pratyusha and Sarada, 2022; Ortiz and Sansinenea, 2023; Kwon *et al*., 2024).

The biological usage of phenolic compounds, which consist of polyphenols, flavonoids, and phenylpropanoids as antioxidants, is spotlighted, and blocking free radicals and reactive oxygen species (ROS) is thus the subject of numerous studies that have been the subject of efforts at extracting them (Pereira *et al*., 2016; Porra and Scheer, 2019). In fact, a number of earlier studies have revealed the correlation with phenolic compounds and antioxidant activities (Fu *et al*., 2011; Lim *et al*., 2024).

Oxidative stress causes diverse disorder pathogenesis, including cardiovascular diseases, neurological diseases, cancer, and respiratory diseases (Rhee, 2006; Pizzino *et al*., 2017). As a result, synthetic antioxidants were invented to prevent oxidation in human health, food decay, and the pharmacology industry (Oktay *et al*., 2003).

However, the side effects of synthetic antioxidants have affected human health; consequently, natural antioxidants have garnered significant interest as alternatives to synthetic antioxidants (Ito *et al*., 1983; Pokorný, 2007). Hence, numerous studies have revealed the correlation between phytochemicals (phenolics, flavonoids, phenylpropanoids, and carotenoids) and antioxidant activity (Kähkönen *et al*., 1999; Moure *et al*., 2001). Based on these considerations, exploration of the natural antioxidants should be conducted for the prevention of disease and treatment associated with oxidative stress.

This study aims to understand the impact of dark and light exposure on compared the antioxidant activity based on differential accumulation of natural pigments and phenolic compounds in adventitious roots of *A. lobatum*.

In the absence of approaches on the influence of light on the accumulation of phenolics and antioxidant activities in the adventitious roots of *A. lobatum*, this study could help maximize the accumulation of phytochemicals in tissue cultures and the potential usage as natural antioxidants.

MATERIALS AND METHODS

1. Sample preparation, adventitious root induction, and light treatment

The seeds of the *Actinostemma lobatum* were collected in

October 2023 from Nonsan, Chungcheongnam-do, Korea. A voucher specimen (NNIBRVP122922) was deposited in the Library of Nakdonggang National Institute of Biological Resources (NNIBR, Sangju, Korea). The sterilized seeds of *A. lobatum* were germinated in a Murashige and Skoog (MS) solid medium (Murashige and Skoog, 1962) at pH 5.8. Seedlings were propagated in a growth chamber using the same medium under 16/8-h light/dark photoperiod at 25℃.

After 4 weeks, a stem segment (0.5 ㎝) was transferred to MS medium with 1.0 ㎎·ℓ$^{-1}$ indole-3-butyric acid (IBA) and kept in the dark to induce adventitious roots. The root samples from 6-week-old grown *A. lobatum* were transferred in a Schenk and Hildebrandt (SH) liquid medium (Schenk and Hildebrandt, 1972) with 1.0 ㎎·ℓ$^{-1}$ IBA and grown on a rotary shaker in the dark for a further 2 weeks.

For light treatment, the acquired adventitious roots were moved into SH liquid medium for 24 h under continuous light at 25℃ from cool white fluorescent; conversely, the control group remained in full darkness for 24 h. After 2 weeks, the samples were harvested, followed by freeze-drying for 72 h. After that, a mortar and pestle were used for grinding the sample, and powder was used for subsequent metabolite analysis and antioxidant activity assays.

2. Assessing chlorophyll and total carotenoid contents from *A. lobatum*

The chlorophyll and total carotenoid contents (TCC) in adventitious roots of *A. lobatum* were assessed according to methods from previous reports with slight modifications (Sumanta *et al*., 2014; Porra and Scheer, 2019). A finely powdered sample was mixed with 99.9% ethanol and incubated at 4℃ for 1 h in the dark. After centrifugation, the supernatant was filtered using PTFE syringe filters, followed by repeating this three times.

Absorbances were read using a UV-vis spectrophotometer (SPECTROstar Nano plate reader, BMG LABTECH., Ortenberg, Baden-Württemberg, Germany), and the contents of chlorophyll and TCC were estimated following equations in Table 1 (Sumanta *et al*., 2014; Porra and Scheer, 2019).

3. Relative quantification of total phenolic and flavonoid contents

The total phenolic contents (TPC) were estimated by adopting the previous protocol, which monitored the reduction rate of Folin-Ciocalteau reagent, from Lim *et al*. (2024). The absorbances were measured at 760 nm. The calibration curve of gallic acid ($y = 0.0014x - 0.0203$, $R^2 = 0.9997$) was used for quantification, and the TPC results were represented in terms of gallic acid equivalent (GAE)·g^{-1} sample dry weight (DW).

The total flavonoid contents (TFC) were determined, followed by the previously reported method, which is based on flavonoid combining characteristics with aluminum, from Lim *et al*. (2024). The absorbances were measured at 415 ㎚. The calibration curve of quercetin ($y = 0.0017x - 0.0054$, $R^2 = 0.9996$) was used for quantification, and the TFC results were presented in terms of quercetin equivalent (QE)·g^{-1} DW.

4. Determination of individual phenolic compounds by high-performance liquid chromatography (HPLC)

Individual phenolic compounds in adventitious roots of *A. lobatum* were determined following the protocol described by Lim *et al*. (2024) with slight modifications. Briefly, 0.1 g of dried sample powder was mixed with 2 ㎖ of 70% MeOH, followed by centrifugation and filtering, and the extracts were directly used for HPLC analysis. The instrument was composed of Agilent 1260 Infinity Ⅱ systems, a C18 column (250 ㎜ × 4.6 ㎜, 5 ㎛, RStech, Daejeon, Korea), and mobile phases of 0.2% acetic acid in distilled water and 99.9% MeOH. The flow rate, column temperature, and wavelength were set to 1 ㎖·min^{-1}, 30℃, and 280 ㎚, respectively. A detailed gradient program was performed following the protocol described by Lim *et al*. (2024).

5. *In vitro* antioxidant assays

In vitro antioxidant activities were estimated using 6 different concentrations of sample (ranging from 62.5, 125, 250, 500,

Table 1. Equation for determining concentrations of chlorophyll *a*, chlorophyll *b*, and total carotenoid.

	Equations
Chlorophyll *a* (mg·g^{-1} DW[1)])	$(13.36 \times A_{664}{}^{2)} - 5.19 \times A_{649}) \times S$
Chlorophyll *b* (mg·g^{-1} DW)	$(27.43 \times A_{649} - 8.12 \times A_{664}) \times S$
Total Carotenoid (mg·g^{-1} DW)	$\{(1000 \times A_{470} - 2.13 \times \text{Chl } a - 97.63 \times \text{Chl } b) / 209\} \times S$

[1)]DW; dry weight of sample, [2)]A_x; absorbance, Chl *a*; chlorophyll *a*, Chl *b*; chlorophyll *b*, S; sample concentraion.

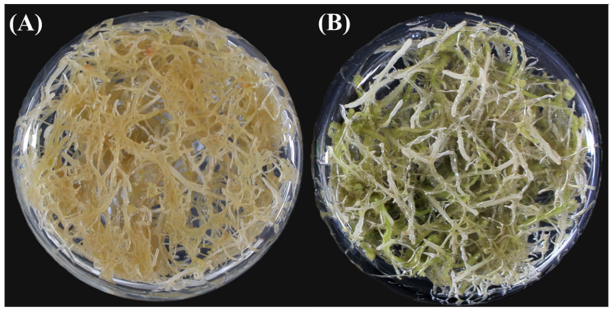

Fig. 1. Adventitious root formation of *A. lobatum*. (A) adventitious roots dark-exposed conditions and (B) adventitious roots light-exposed conditions.

1000, to 2,000 $\mu g \cdot m\ell^{-1}$) and ascorbic acid as a positive control. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity in adventitious roots of *A. lobatum* were performed following a protocol from an earlier report by Lim *et al.* (2024). The required absolute 50% inhibitory concentration (IC_{50}) was calculated based on dose-response curves plotted using absorbance data and expressed in $mg \cdot m\ell^{-1}$. Reducing power was assessed by monitoring the conversion of ferric ion, and methodology was adopted from Lim *et al.* (2024).

6. Statistical analysis

All values were expressed as the mean ± standard deviation (SD) based on triplicate data. Statistical analysis was performed by using SPSS 20 (SPSS Inc., Chicago, IL, USA), and the significances were determined by the *t*-test and Duncan's Multiple Range Test (DMRT) at the 5% level ($p < 0.05$).

RESULTS

1. Assessment of natural pigment under dark and light exposure

Chlorophyll contents from the adventitious roots of *A. lobatum* were significantly different under the dark and light-exposed cultures. The adventitious root phenotypic color appeared to be a light brown hue in the dark-grown culture, whereas the light-grown culture showed a greenish color (Fig. 1).

Although there is no significant difference between the dark and light treatment groups in the contents of chlorophyll *b*, the light-exposed cultures significantly accumulated about 2.0 times higher than dark-exposed cultures in chlorophyll *a* ($p < 0.05$) (Table 2). In addition, the light-exposed cultures (0.047 ± 0.001 $mg \cdot g^{-1}$ DW) contained significantly higher TCC than in the dark-exposed cultures (0.026 ± 0.003 $mg \cdot g^{-1}$ DW) (Table 2). From these results, the exposure to dark and light influenced significant changes to natural pigment in the adventitious roots of *A. lobatum*.

2. Quantification of TPC and TFC contents from *A. lobatum*

As shown in Table 3, light-exposed adventitious roots had a greater accumulation of TPC and TFC than dark-exposed adventitious roots with significant differences. Specifically, under light conditions, the TPC (11.273 ± 0.291 GAE·$mg \cdot g^{-1}$ DW) and TFC (9.943 ± 0.28 QE·$mg \cdot g^{-1}$ DW) achieved 1.47 and 2.05 times higher than under the dark conditions (7.665 ± 0.291 GAE·$mg \cdot g^{-1}$ DW and 4.855 ± 0.074 QE·$mg \cdot g^{-1}$ DW, respectively). These results suggest that light significantly influenced the phenolic and flavonoid contents in the adventitious roots of *A. lobatum*.

3. Determination of individual phenolic contents

Subsequently, we identified and quantified individual phenolic compounds from the adventitious roots of *A. lobatum*. Among the identified compounds, (-)-epicatechin, benzoic acid, and kaempferol were not identified as having significant differences;

Table 3. Total phenolic and flavonoid contents from dark and light treated adventitious roots of *A. lobatum*

Treatments	TPC[1)] (GAE·$mg \cdot g^{-1}$ DW)	TFC[2)] (QE·$mg \cdot g^{-1}$ DW)
Dark	7.665±0.291	4.855±0.074
Light	11.273±0.291*	9.943±0.28*

[1)]TPC; total phenolic content, GAE; gallic acid equivalent, DW; dry weight of sample; [2)]TFC; total flavonoid content, QE; quercetin equivalent. Values are the means ± SD, and the statistically significant differences were determined by *t*-test at the 5% level (*$p < 0.05$).

Table 2. Effects of dark and light on the accumulation of chlorophyll *a*, *b*, and total carotenoid contents from *A. lobatum*.

	Chlorophyll *a* Contents ($mg \cdot g^{-1}$ DW[1)])	Chlorophyll *b* Contents ($mg \cdot g^{-1}$ DW)	TCC[3)] ($mg \cdot g^{-1}$ DW)
Dark	0.072±0.001	0.051±0.004	0.026±0.003
Light	0.136±0.001*	0.057±0.003[ns2)]	0.047±0.001*

[1)]DW; dry weight of sample, [2)]ns; not significant, [3)]TCC; total carotenoid content. Values are the means ± SD, and the statistically significant differences were determined by *t*-test at the 5% level (*$p < 0.05$).

Table 4. Accumulation of individual phenolic compounds from dark and light treated adventitious roots of *A. lobatum*.

Treatments	Phenolic Compound Contents (㎎·g^{-1} DW[1)])				
	(-)-Epicatechin	Sinapic acid	Benzoic acid	Rutin	Kaempferol
Dark	0.063±0.002	ND	0.004±0.001	0.329±0.065	0.018±0.000
Light	0.069±0.004^{ns}	0.09±0.004*	0.004±0.001^{ns}	2.136±0.491*	0.018±0.000^{ns}

[1)]DW; dry weight of sample; ND; not-detected, ns; not significant. Values are the means ± SD, and the statistically significant differences were determined by *t*-test at the 5% level ($^{*}p < 0.05$).

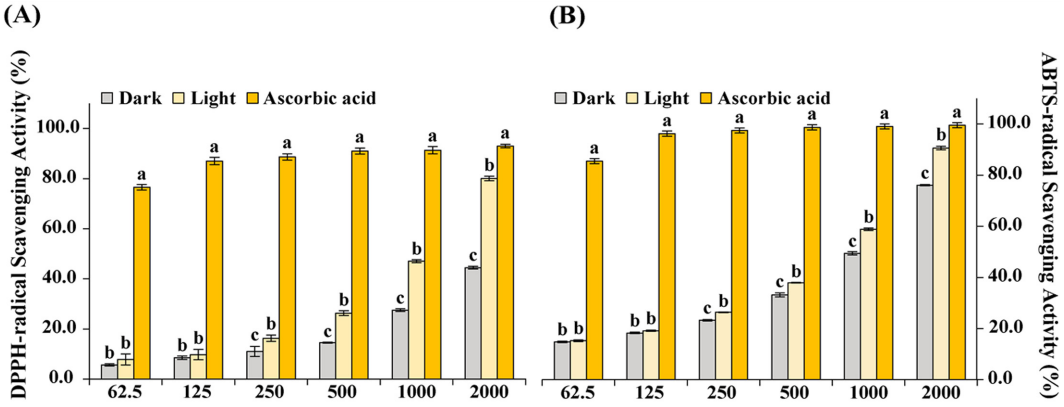

Fig. 2. *In vitro* antioxidant activities from dark and light treated adventitious roots of *A. lobatum*. Values are the means ± SD, and (A) DPPH-radical scavenging activity, and (B) ABTS-radical scavenging activity. X-axis and Y-axis represent the concentration of extracts (㎍·㎖$^{-1}$) and inhibition activity (%), respectively. The values with the letter "a" represent the highest using Duncan's Multiple Range Test (DMRT, $p < 0.05$).

however, sinapic acid that belongs to phenylpropanoid was not detected in the dark-exposed sample, whereas the light-exposed sample was 0.09 ± 0.004 ㎎·g^{-1} DW. Interestingly, rutin, which belongs to flavonoids, was accumulated higher in light-exposed roots of *A. lobatum* (2.136 ± 0.491 ㎎·g^{-1} DW) than in dark-exposed conditions (0.329 ± 0.065 ㎎·g^{-1} DW).

Although (-)-epicatechin and kaempferol were not confirmed to have a significant difference between dark and light conditions, we can assume that light could induce the accumulation of phenylpropanoid and flavonoids in adventitious roots of *A. lobatum*.

4. *In vitro* antioxidant activities

The DPPH, ABTS, and reducing power were efficient assays to evaluate antioxidant activity in a fast and accurate manner and were performed to estimate the effect of dark and light on antioxidant activity from the adventitious roots of *A. lobatum*.

First, the increase in concentration led to an increase in scavenging activity in both, and the radical scavenging activity of DPPH and ABTS was greater in exposed light samples. Although there were no statistical differences between the dark and light-exposed groups at concentrations of 62.5 and 125 ㎍·㎖$^{-1}$, statistical differences could be seen at a concentration of 250 ㎍·㎖$^{-1}$. (Fig. 2).

Table 5. IC_{50} value of DPPH and ABTS radical from dark and light treated adventitious roots of *A. lobatum*.

Treatments	IC_{50} value of DPPH (㎎·㎖$^{-1}$)	IC_{50} value of ABTS (㎎·㎖$^{-1}$)
Dark	2.235±0.013	1.109±0.004
Light	1.157±0.013*	0.88±0.006*

Values are the means ± SD, and the statistically significant differences were determined by *t*-test at the 5% level ($^{*}p < 0.05$).

At a concentration of 2,000 ㎍·㎖$^{-1}$, in the DPPH assay, light-exposed *A. lobatum* roots (80.07 ± 0.93%) achieved significantly higher activity than dark-exposed (44.49 ± 0.56%) (Fig. 2A), and similarly in the ABTS assay, exposure to the light (90.56 ± 0.67%) was significantly more influential to scavenging activity than exposure to the dark (76.07 ± 0.29%) (Fig. 2B).

As shown in Table 5, both in DPPH and ABTS, the IC_{50} value of light-exposed samples (1.157 ± 0.013 ㎎·㎖$^{-1}$ and 0.88 ± 0.006 ㎎·㎖$^{-1}$, respectively) was estimated to be higher in

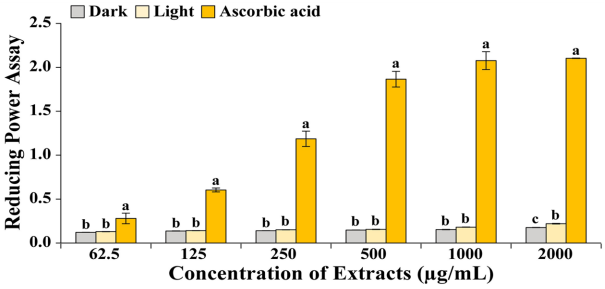

Fig. 3. Effects of dark and light on the antioxidant activity of reducing power from adventitious roots of *A. lobatum*. Results are represented by means ± SD, and the letter "a" shows the highest by One-way ANOVA using Duncan's Multiple Range Test (DMRT, $p < 0.05$).

efficiency than dark-exposed samples (2.235 ± 0.013 $mg \cdot m\ell^{-1}$ and 1.109 ± 0.004 $mg \cdot m\ell^{-1}$).

Due to the use of crude extracts, the low sample concentrations likely contained relatively fewer biologically active metabolites. Consequently, no significant differences were observed at lower concentrations. However, a concentration-dependent increase in activity was evident. Notably, at a concentration of 2,000 $\mu g \cdot m\ell^{-1}$, light exposure significantly enhanced the reducing power, indicating a higher degree of Fe^{3+} to Fe^{2+} conversion.

Specifically, the adventitious roots of *A. lobatum* under light conditions (0.22 ± 0.01) showed higher efficiency than under dark conditions (0.18 ± 0.01) (Fig. 3).

Based on these results, the exposure of the light to adventitious roots of *A. lobatum* significantly influenced the accumulation of phytochemicals, such as chlorophylls, carotenoids, phenolics, flavonoids, and phenylpropanoids, and at the same time, resulted in a higher efficiency of antioxidant activity.

DISCUSSION

Environmental factors significantly influenced not only the generation but also the accumulation of metabolites from plants (Park *et al*., 2024). In fact, the strategies regulating light have been reported to increase the metabolites in plants (Do *et al*., 2023; Park *et al*., 2024). In addition, numerous previous studies have revealed the superiority of inducing adventitious root cultures to produce plant secondary metabolites (Rahmat and Kang, 2019; Khanam *et al*., 2022).

In this study, we aimed to discover that dark and light caused prominent effects on the accumulation of bioactive compounds, following higher antioxidant activities in *A. lobatum* adventitious root cultures.

As natural pigments, chlorophylls and carotenoids are well known for various biological activities. In our results, under the light conditions, significantly enhanced chlorophyll and carotenoid contents in adventitious roots of *A. lobatum* by regulating light-mediated photosynthesis.

Park *et al*. (2024) demonstrated that the light condition definitely induced the chlorophyll, carotenoid, and phenylpropanoid biosynthetic pathways in hairy roots of *A. officinalis*, which were consistent with our results. Moreover, Lee *et al*. (2023) reported that the light treatment significantly impacted the production of rosmaric acid, TPC, antioxidant activities, and antimicrobial activities in *Perilla frutescens*. Namely, the light treatments are anticipated to induce the production of phyto-chemicals and beneficial effects in coincidence.

A previous study reported the bioactivity of chlorophyll, such as anti-cancer, interruption of cardiovascular disease, and other chronic diseases (Pareek *et al*., 2017). Carotenoids defend against oxidative damage to chlorophyll and assist the chlorophyll as photosynthetic pigments (Vechtel *et al*., 1992; Sumanta *et al*., 2014). They should be consumed via diet due to their essential features for humans, and numerous studies have revealed the excellent usage of carotenoids as antioxidants (Muller and Bohm, 2011; Jiří *et al*., 2024).

In other words, exposure to light could lead to the significant accumulation of chlorophyll and carotenoids, resulting in improved photosynthetic systems and their bioactivity.

According to previous reports, they suggested that the light strongly influenced the activation of phenolic biosynthetic pathways (Liu *et al*., 2021; Yun *et al*., 2022). Light exposure roots of *A. lobatum* contained significantly higher secondary metabolites (carotenoids, phenolics, and flavonoids), which are the main scavengers reacting to oxidative stress (Moure *et al*., 2001).

Carotenoids, phenolics, and flavonoids have had their bioactivity proven by previous reports (Bungala *et al*., 2024; Jiří *et al*., 2024; Lim *et al*., 2024). In detail, sinapic acid and rutin were significantly accumulated under light than dark conditions. Among them, rutin was achieved at approximately 6.49 times higher in light conditions, and Prasad *et al*. (2019) demonstrated the various bioactive properties (antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, hepatoprotective, and antidiabetic activity) and the usage in nutraceuticals of rutin.

Light treatment in adventitious roots of *A. lobatum* considerably contributed to the production of chlorophyll, total carotenoid,

phenolic, and flavonoid, and the improved antioxidant activities might be due to the increase of secondary metabolites (Pizzino *et al*., 2017). Indeed, several previous reports revealed a remarkable correlation between antioxidant activities and total phenolic contents in plants (Kähkönen *et al*., 1999; Fu *et al*., 2011).

In conclusion, the light treatment substantially promoted the biosynthesis of phytochemicals and augmented antioxidant responses, indicating its pivotal role in metabolic regulation and antioxidant activity in the adventitious roots of *A. lobatum*.

This strategy might be helpful for the enhancement of valuable bioactive compounds in the adventitious roots of *A. lobatum* and utilized for the optimization of in vitro cultures in the adventitious roots of *A. lobatum*, contributing to the pharmaceutical and nutraceutical industries.

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