



Effects of light-emitting diode (LED) lights on growth and bioactive compound contents in *Andrographis paniculata* (Burm.f.) Nees

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Abstract

Background and Objective: *Andrographis paniculata* (Burm.f.) Nees is a herb that act as agents of anti-inflammatory, reduce fever and alleviate respiratory symptoms. However, there is an inadequacy of information on the influence of light quality on the growth and accumulation of secondary metabolites in *A. paniculata*. Therefore, this research aimed to study the effects of LED lights on growth and bioactive compounds in *A. paniculata*.

Methodology: The *A. paniculata* seeds were grown in a 104-hole seedling tray, and then 30-day-old seedlings were transplanted into a grow bag measuring 8 × 15 cm. The seedlings were grown in a greenhouse under natural light and supplemented with different LED lights from 18.00 to 20.00 p.m., including fluorescent (control), blue, and red LED lights. The light intensity was 100 μmol/m²/s. Plants were harvested 3 months after transplanting. This experiment was conducted by a completely randomized design (CRD) with ten replications. Data were analyzed with one-way ANOVA. Treatment mean ± standard deviations (SD) differences assessed via Duncan's Multiple Range Test (DMRT).

Main Results: Red LED light-induced flowering of *A. paniculata* was 59.00 ± 0.50 days after transplanting (P < 0.05) and gave the highest yield of the leaf (245.61 ± 0.30 g/plant) compared to other treatments (P < 0.05). Additionally, red and blue LED lights increased total chlorophyll content in the leaves compared to the control group (P < 0.05). Furthermore,

blue LED light stimulated the production of the highest andrographolide content (158.57 ± 1.73 mg/g dry weight) and 14-deoxy-11,12-didehydroandrographolide content (0.35 ± 0.02 mg/g dry weight), respectively ($P < 0.05$).

Conclusions: Red LED light promoted flowering and yield of the leaf, while blue light increased the number of bioactive compounds, including andrographolide and 14-deoxy-11,12-didehydroandrographolide contents in *A. paniculata*. These findings highlight the potential of LED lighting as a tool for optimizing the cultivation and enhancing the medicinal value of *A. paniculata*.

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INTRODUCTION

In Thailand, *Andrographis paniculata* is referred to as Fa-Tha-Lai-Jone, which is included in Thailand's National List of Essential Medicines. It has been used in traditional medicine for treating colds, fevers, and respiratory symptoms (Jarukamjorn and Nemoto, 2008). Nowadays, it serves as an alternative for the treatment of COVID-19 (Shi *et al.*, 2020; Intharuksa *et al.*, 2022). Andrographolide is a diterpene lactone compound that is the major bioactive compound in *A. paniculata* and has a strong bitter taste (Sharma *et al.*, 2017). Several studies have confirmed its pharmaceutical benefits, such as anti-inflammatory, antioxidant, and immune-boosting properties (Hossain *et al.*, 2014; Dai *et al.*, 2019). The pharmacological properties of *A. paniculata* have been intensely investigated in the past year (Xu *et al.*, 2019; Mussard *et al.*, 2020). However, the content of andrographolide varies according to genetic and environmental conditions (Chutimanukul *et al.*, 2022).

Light is one of the most important environmental factors that affect plant photosynthesis, growth, development, and secondary metabolism (Livadariu *et al.*, 2023). The visible spectrum has a wavelength

range of 380 to 780 nm, with 400–700 nm being the most effective wavelength for plant photosynthesis (Ma *et al.*, 2021). Currently, light-emitting diode (LED) technology is used for growing plants in greenhouses because LED allows adjustable light intensity, duration time, and light wavelength (Trivellini *et al.*, 2023). It has previously been reported that red LED light enhances the growth and development of artichoke seedlings (Rabara *et al.*, 2017). Bottiglione *et al.* (2024) reported that red light increased the elongation of lentil seedlings, while blue light promoted bioactive compounds and antioxidant activities. Moreover, Panjai *et al.* (2021) reported that red light accelerated the color development, total phenolics, and total flavonoids of tomatoes. Similarly, Pola *et al.* (2020) reported that red light enhanced color development and lycopene accumulation, while blue light promoted total phenolics and vitamin C of green chilli. As a result, using LED light to grow plants could be considered an alternative technique that was highly effective in producing crops and stimulating the bioactive compounds in plants (Karimi *et al.*, 2022). However, there is limited information on the effects of light quality on the growth and accumulation of

secondary metabolites in *A. paniculata*. Therefore, the objective of this study was to investigate the effects of LED lights on growth and bioactive compounds in *A. paniculata*.

MATERIALS AND METHODS

Plant Material

The *A. paniculata* seeds were grown in a 104-hole seedling tray, and then 30-day-old seedlings were transplanted into a grow bag measuring 8 × 15 cm. The seedlings were grown in a greenhouse at King Mongkut's University of Technology Thonburi, Thailand. The average temperature was 30.32 ± 0.54°C, and the relative humidity was 77.00 ± 1.81%.

Experimental Treatments

The seedlings were grown under natural light and supplemented with different LED lights at a light intensity of 100 μmol/m²/s from 6.00 to 8.00 p.m. The treatments include:

Treatment 1: Fluorescent (control)

Treatment 2: Blue LED

Treatment 3: Red LED

An organic fertilizer (85% chicken manure and 15% sugar cane bagasse) was added at 125 g/bag every 30 days, and drip irrigation was applied twice a day. Plants were harvested 3 months after transplanting.

Data Collection

Flowering measurement

The flowering was determined by counting the time after transplanting to the primary inflorescences produced (day).

Yield of leaf

The yield of the leaf was determined by the average weight of the leaf per plant with an electronic balance (g/plant).

Total chlorophyll content

Total chlorophyll content was measured by the

method of Moran (1982). The fresh leaf sample (0.5 g) was weighed and then added N,N-Dimethylformamide 10 mL. The sample was stored in the dark at 4°C for 24 h. The sample was filtered using Whatman® filter paper No. 1. The absorbances were measured at 664 and 647 nm using a spectrophotometer (UV-1800; Shimadzu Co., Kyoto, Japan). The chlorophyll contents were expressed on a fresh weight basis in mg/g fresh weight.

Andrographolide and 14-deoxy-11,12-didehydroandrographolide contents

The dried sample (1 g) was weighed, and 50% methanol (25 mL) was added. The sample was put in an ultrasonic bath for 30 min. The sample was filtered using Whatman® filter paper No. 1. The supernatant was evaporated using a rotary evaporator. The crude extract was dissolved in 50% methanol (1 mL), filtered through a 0.45 μm filter membrane, and 20 μL sample was injected into high-performance liquid chromatography (HPLC) (Agilent 1200 Series Rapid Resolution LC system). The andrographolide and 14-deoxy-11,12-didehydroandrographolide contents were analyzed according to the method of Dalawai *et al.* (2021). The mobile phase involved a solvent of water: acetonitrile (60:40) at a constant flow rate of 1.0 mL/min for 15 min. The column temperature was maintained at 25°C. The compounds were identified by comparing their retention times with andrographolide and 14-deoxy-11,12-didehydroandrographolide standards at 223 nm.

Statistical Analysis

All collated data were analyzed using a completely randomized design (CRD) in ten replicates per treatment. The report of the data was given as mean ± SD. Duncan's Multiple Range Test (DMRT) determined significant differences (P < 0.05) using statistical SAS software version 9.0 (SAS Institute; Cary, NC, USA).

RESULTS AND DISCUSSION

Effect of LED Lights on Flowering and Yield of Leaf

The effect of LED light treatments on the flowering and yield of leaves of *A. paniculata* are shown in Table 1. The results found that red LED light significantly induced the flowering of *A. paniculata* compared to other treatments ($P < 0.05$). Moreover, red light also showed the highest yield of the leaf (245.61 ± 0.30 g/plant), followed by fluorescent light (204.51 ± 0.70 g/plant) and blue LED light (161.35 ± 0.94 g/plant), respectively ($P < 0.05$). Red LED light influenced flowering and yield of the leaf not directly through photosynthesis but rather through its effects on plant photoreceptors. Red LED light is absorbed by phytochrome, which is a plant photoreceptor (Qiu *et al.*, 2023). Phytochrome exists in two forms,

including Pr (inactive form) and Pfr (active form). The Pr is converted into the Pfr when plants are exposed to red light. Red LED light helped regulate various growth processes, including stem elongation, leaf expansion, and flowering (Kusuma and Bugbee, 2021). Moreover, red LED light is perfectly absorbed by chlorophyll molecules for driving the photosynthetic processes, which indirectly supports flowering and increases the yield of plants (Dou *et al.*, 2017). Similarly, Cao *et al.* (2016) reported that the use of red LED at night promoted plant height, flowering, and yield of tomatoes. Karimi *et al.* (2022) reported that red LED light promoted significant increases in the growth and flowering of *Hypericum perforatum* L. (cv. Topas) compared with blue LED light.

Table 1 Days to flowering and yield of the leaf of *A. paniculata* (Burm.f.) Nees on fluorescent (control), blue, and red LED at a light intensity of $100 \mu\text{mol}/\text{m}^2/\text{s}$ from 6.00 to 8.00 p.m.

Treatments	Days to flowering (days after transplanting)	Yield of the leaf (g/plant)
Fluorescent	81.00 ± 0.30^a	204.51 ± 0.70^b
Blue LED	81.00 ± 0.40^a	161.35 ± 0.94^c
Red LED	59.00 ± 0.50^b	245.61 ± 0.30^a

Note: Means within the same column followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$). Values are presented as mean \pm standard deviation.

However, blue LED light produced a significantly lower yield than red LED light because it had a shorter wavelength and higher energy (Kuse *et al.*, 2014). The higher energy of blue light could create an excess of energy within the plant that leads to photoinhibition of photosynthesis. Moreover, this excess of energy might end up in the production of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide, and singlet oxygen (Sachdev *et al.*, 2021). These ROS had the ability to damage the Photosystem II (PSII) reaction center, upsetting the critically important electron transport chain (Wang *et al.*, 2018). This disruption limits growth and development by decreasing the plant's ability to efficiently convert light energy into chemical

energy (ATP and NADPH) (Li *et al.*, 2023), which eventually results in lower yields. Furthermore, blue light could have varying effects on flowering and yield in plants in different ways, depending on the intensity of the light and the type of plant. Many researchers have reported that blue light could promote flowering in some plants while suppressing flowering and yield in others. Blue LED light promoted chrysanthemum flowering and increased the number of flower buds (Park and Jeong, 2020). Similarly, Ye *et al.* (2021) reported that blue light improved strawberry flowering. On the other hand, lettuce with a high blue light ratio showed less biomass accumulation, shorter stems, and fewer leaves (Kong and Nemali, 2021).

Although excessive amounts of blue light inhibited plant growth and biomass production because photosynthesis did not utilize all of the energy, a low dose of blue light is required to complement red light for the most effective achievable plant growth (Cope and Bugbee, 2013). The negative effects of blue light might be minimized by combining red and blue lights in particular ratios based on plant species, growth stage, and environmental conditions. For commercial applications, LED light could supplement or replace natural sunlight in controlled environments. Applying LED lighting is especially well-suited for vertical farming systems, where space is limited. Optimizing the light spectrum and light intensity can increase crop yields in vertical farms while decreasing energy consumption.

Effect of LED Lights on Total Chlorophyll Contents

Our findings demonstrate that different light qualities had variable effects on the total chlorophyll content of *A. paniculata* (Figure 1). The total chlorophyll contents were the highest in *A. paniculata* exposed to red LED light (6.77 ± 0.06 mg/g FW), while plants treated with fluorescent light had the lowest total chlorophyll contents (5.97 ± 0.15 mg/g FW; $P < 0.05$). Red LED light influenced the production and function

of both chlorophyll a and chlorophyll b. Red LED light stimulated the synthesis of chlorophyll content and enhanced the ability of plants to capture light energy for photosynthesis, boosting overall plant growth and development. Moreover, red LED light affected phytochrome, which regulated various growth processes. Phytochrome activation by red light promoted chlorophyll production, including flowering and yield of plants (Kusuma and Bugbee, 2021). This explanation supported our results, which showed that red light motivated flowering and enhanced the yield of the leaf of *A. paniculata*. Moreover, plants supplemented with blue LED light also showed an increase in total chlorophyll content. This might be due to blue light stimulating chlorophyll synthesis, particularly chlorophyll b for the photosynthetic process. Accordingly, red and blue LED lights had the greatest influence on the photosynthesis rate in that they had an impact on the accumulation of total chlorophyll contents in plants (Dou *et al.*, 2017; Naznin *et al.*, 2019).

Nevertheless, the effects of these variations in the total amount of chlorophyll extended beyond photosynthesis. The increase in total chlorophyll contents of *A. paniculata* after exposure to LED lights might lead to increased bioactive compounds and biomass accumulation in plants (Baidya *et al.*, 2021;

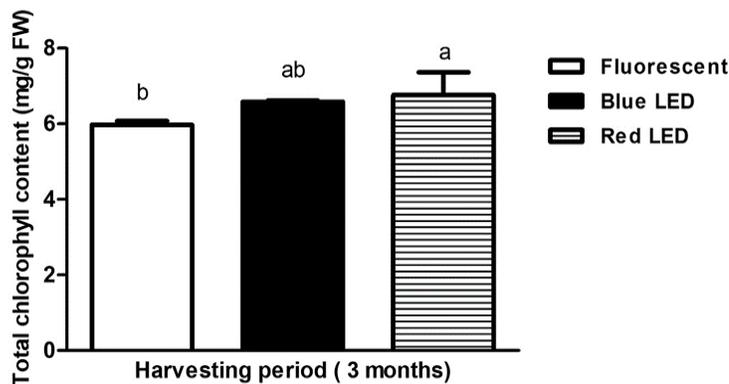


Figure 1 Total chlorophyll contents (mg/g fresh weight) of *A. paniculata* (Burm.f.) Nees on fluorescent (control), blue, and red LED at a light intensity of $100 \mu\text{mol}/\text{m}^2/\text{s}$ from 6.00 to 8.00 p.m. The different letters indicated significant differences as determined by Duncan’s Multiple Range Test (DMRT) at $P < 0.05$.

Tangtragoon *et al.*, 2023). This is supported by research on various medicinal plants such as *Withania somnifera* (Adil *et al.*, 2019) and *Hedyotis corymbosa* (L.) Lam (Le *et al.*, 2022). Additionally, the amount of chlorophyll can be used as an indicator of plant stress tolerance. Higher chlorophyll content in plants frequently indicates their resistance to environmental factors such as drought and salinity (Ma *et al.*, 2020). Therefore, the elevated chlorophyll content in *A. paniculata* under LED light might suggest improved stress tolerance, which could be beneficial for optimizing cultivation practices to produce medicinal plants with high-quality.

Effect of LED Lights on Andrographolide and 14-Deoxy-11,12-Didehydroandrographolide Contents

The effect of different LED light treatments on andrographolide and 14-deoxy-11,12-didehydroandrographolide contents of *A. paniculata* are shown in Figures 2A–2B. The content of andrographolide of *A. paniculata* in the blue LED light treatment (158.57 ± 1.73 mg/g DW) was significantly higher than the

control group (143.34 ± 2.62 mg/g DW) ($P < 0.05$). Moreover, blue LED light treatment showed the highest content of 14-deoxy-11,12-didehydroandrographolide (0.35 ± 0.02 mg/g DW) ($P < 0.05$). LED light had a greater impact on the growth and development of plants, which could indirectly influence the synthesis of secondary metabolites via glycolysis, the Krebs cycle, and the pentose phosphate pathway (Aharoni and Galili, 2011). *A. paniculata* is mainly attributed to the presence of andrographolides, which is a group of diterpenoid lactones (Murthy and Dalawai, 2021). The synthetic pathways of diterpenoid lactones seem to proceed by both mevalonic acid (MVA) and methyl erythritol phosphate (MEP) pathways (Srivastava and Akhila, 2010). Moreover, blue LED light improved photosynthetic efficiency by increasing the total chlorophyll content of *A. paniculata*, which could lead to increased availability of metabolic precursors for boosting the synthesis of secondary metabolite, including diterpenoid lactones like andrographolide and 14-deoxy-11,12-didehydroandrographolide (Landi *et al.*, 2020).

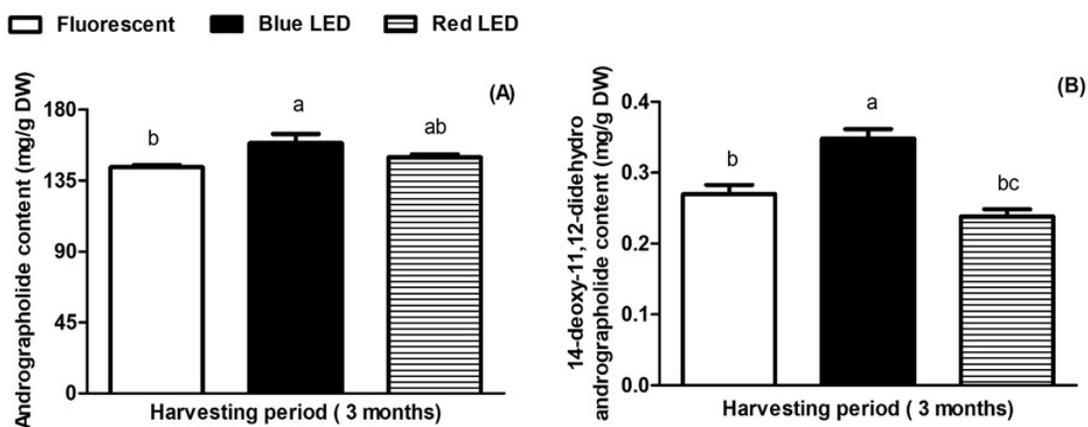


Figure 2 Andrographolide (A) and 14-deoxy-11,12-didehydroandrographolide contents (B) (mg/g dry weight) of *A. paniculata* (Burm.f.) Nees on fluorescent (control), blue, and red LED at a light intensity of $100 \mu\text{mol}/\text{m}^2/\text{s}$ from 6.00 to 8.00 p.m. The different letters indicated significant differences as determined by Duncan’s Multiple Range Test (DMRT) at $P < 0.05$.

Previous studies have confirmed that LED light promotes the synthesis of secondary metabolites (Zhang *et al.*, 2020; Bungala *et al.*, 2024). According to Alrifai *et al.* (2019), blue LED light promoted the accumulation of isoflavonoids and astragalosides in microgreen vegetables. Similarly, blue LED light enhanced the production of rosmarinic acid and eugenol in *Ocimum basilicum* callus (Nadeem *et al.*, 2019). The enhanced production of andrographolide and 14-deoxy-11,12-didehydroandrographolide under blue LED light significantly improved the pharmaceutical value of *A. paniculata* due to achieving greater amounts of active ingredients, allowing for the use of less plant material for extraction.

However, the growth of *A. paniculata* under LED lighting is not seasonally dependent and can produce raw materials year-round to support market demand, making LED lights a potentially cost-effective and long-term sustainable solution for commercial large-scale cultivation. The initial investment in LED lighting may be higher than traditional fluorescent lighting, but long-term savings are significant. LEDs use a lot less energy because they turn a larger percentage of electrical energy into light, whereas fluorescent lights waste a lot of energy as heat. Additionally, LEDs have a longer lifespan than

fluorescent lights, which minimizes the need for frequent replacements and lowers maintenance expenses. Furthermore, LED lighting can not only increase yield but also improve the quality of crops. Thus, this technology led to an increase in the number of harvests per year, high quality, and increased income.

CONCLUSIONS

The supplementation of red LED light could simultaneously promote the flowering and yield of leaf. Moreover, blue LED light increased the number of bioactive compounds, including andrographolide and 14-deoxy-11,12-didehydroandrographolide contents in *A. paniculata*. Furthermore, further research is needed to investigate the optimization of light intensity and quality by carefully experimenting with various blue-to-red ratio combinations within the LED spectrum to maximize the production of secondary metabolites and scaling up experiments for commercial applications.

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