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Effects of LED Lighting Technology on Morphology, Antioxidant activity, and the Bioactive Compounds Accumulation of *Anoectochilus burmannicus* in the Greenhouse System

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Abstract

Anoectochilus burmannicus is valued for medicinal and ornamental purposes. It is characterized by beautiful foliage with attractively red-purple with bronze-colored veins blotches. Kinsenoside is an active compound in *A. burmannicus* which provided anti-inflammation properties, anti-obesity, and anti-diabetes properties. This study aimed to investigate the effects of light-emitting diode (LED) light quality on growth, photosynthetic pigment content, antioxidant activities, and the accumulation of bioactive compounds (polysaccharides, phenolics, and flavonoids) in *A. burmannicus*. Plants of *A. burmannicus* were grown under 6 different LED lighting in the greenhouse system for 60 days. The 6 treatments were blue light (BL, 24 h), cool light (CL, 24 h), natural light (NL, 12 h), red:blue 1:1 light (RBL, 24 h), red light (RL, 24 h), and warm light (WL, 24 h). The results showed that *A. burmannicus* plants have a positive effect on the growth and bioactive compounds under LED lights (24 h) technology compared to natural light (12 h). The WL treatment had the most positive effect on morphological parameters, while the BL treatment showed an enhancement in fresh weight and dry weight. NL treatment is increased the Chlorophyll a and Chlorophyll b content. The Carotenoid content increased in the RBL treatment phenolics content was considered tent were considered to be superior to the BL treatment. The total flavonoid content is increased in the RL and RBL treatments. The antioxidant activity of ABTS was significantly higher in the RBL treatment. This indicates that BL is required for the normal growth, biomass, and accumulation of polysaccharides and phenolic of *A. burmannicus*, while RBL is enhanced the accumulation of flavonoids and antioxidant activity. This technology is conducive to achieving large-scale sustainable production of high-quality medicinal plant materials.

Keywords: *Anoectochilus burmannicus*, Antioxidant activity, Bioactive compounds, Continuous lighting, LED lighting technology

Introduction

A genus of small terrestrial orchids, *Anoectochilus* is a member of the jewel orchids group in the Orchidaceae found in tropical and subtropical rain forests. These orchids are traditionally used extensively in China and many Asian countries due to their medicinal properties and therapeutic benefits. They are employed for treatment in different systems, such as the circulatory system, liver disease, kidney disease, inhibiting cancer cells, and anti-inflammatory [1-3]. Various nutraceutical health products have been produced from *Anoectochilus* because of its healthcare effects [4].

A. burmannicus is found in evergreen forests and hill evergreen forests in shady; native to China (Yunnan), Myanmar, Laos, Peninsular Malaysia, and Thailand. In Thailand is found in Chiang Mai, Chiang Rai, Tak, Loei, and Kanchanaburi [5,6]. It is characterized by beautiful foliage that possesses distinctive velvety foliage with attractively red-purple with bronze-colored veins blotches. The secondary metabolite of *A. burmannicus* includes polysaccharides, phenolic, and flavonoids and shows antioxidant properties. Kinsenoside is the active compound in *A. burmannicus* that provided anti-inflammation property, anti-obesity, and anti-diabetes properties [7]. *A. burmannicus* has been traditionally harvested mainly from wild populations. It is increasingly in demand for both medicinal and ornamental purposes. However, wild populations of *A. burmannicus* in Thailand are facing extinction due to overexploitation, specific growth

conditions, slow growth rate, and increasingly severe climate change problems [8]. Artificial cultivation technology is a potential way to meet the growing market demand for *A. burmannicus* and to avoid the population's extinction in the wild.

The quantity and quality of light is the most important factor affecting plant growth and the accumulation of plant secondary metabolites. The Radiation within the 400 - 700 nm band of photosynthetically active radiation (PAR) regulates the photochemical reactions, converting light energy into chemical energy, through the synthesis of ATP and NADPH used to assemble carbon atoms in organic molecules in the Calvin cycle and the synthesis of amino acids and lipids [9]. A useful spectrum for photosynthesis in the PAR range is perceived through photosynthetic pigments, chlorophylls, lutein, zeaxanthin, lycopene, and carotenoids as β-carotene, which respond to regular wavelengths included in this range. The light-harvesting complex in the thylakoids of the chloroplast consisting of chlorophyll a and chlorophyll b, exhibited the maximum absorption peak at 430, 662 and 453, 642 nm, respectively [10]. Carotenoids are complementary photosynthetic pigments, harvesting and transferring light energy to chlorophylls. Its uptake peak in the range of 400 - 500 nm, plays an important role in protecting plants from oxidative stress, by the dissipation of excess light energy absorption by photosystems [11].

The application of artificial light is more useful than using chemicals to manage plant architecture and simultaneously reduce environmental impacts [12]. The yield and plant biomass increased by supplemental light was largely determined by the daily integral light received by plants. Long photoperiods of lighting have an economic advantage over a short period of lighting in achieving the same DLI with less light fixtures (capital) costs. More recently, the trend towards continuous (24 h) supplemental lighting has received much interest due to the potential for increased yield and its inherent reduction in energy [13-16].

The rapid development of lighting technologies using light-emitting diodes (LEDs) has led to increasing the application of this technology for lighting in closed-plant production systems [17]. Various effects of LED light on morphology and development were reported in many plant species, which differ in their responses to light quality. For monochromatic light, blue and red lights are the major wavelengths perceived by plant photoreceptors. The photo responses are wavelength-dependent reactions [18], which take place with blue light in the region of 400 - 500 nm and with red light in that of 600 - 700 nm. Furthermore, it is noteworthy that blue and red lights can affect plant morphology, physiology and development, photosynthesis, and primary and secondary metabolism [19]. Although there is a large literature on the effect of blue: red light on the nutritional traits of plants, its role on the physiological, biochemical, and nutritional traits of wheatgrass, sprouts, and microgreens is still unclear or unavailable for most plant species [20,21].

Anoectochilus is suitable for cultivation under low light intensity with a photosynthetic photon flux of 30 - 50 m mole m^{-2} s^{-1} for each growth for both growth and the production of secondary metabolites [20]. *A. roxburghii* has increased levels of chloroplasts, grana, and grana lamellae, and higher POD and SOD activities to adapt to light conditions under 30 % irradiance [22]. Wang *et al*. [23] demonstrated the effects of supplemental lighting with different light qualities on the growth and secondary metabolite content of *A. roxburghii*. The result was shown that blue light (BL) treatment was contributing to promoting the growth and accumulation of secondary metabolites (total flavonoids, total polyphenols); yellow light (YL) treatment significantly enhanced the content of soluble sugar and polysaccharides over the control. The authors didn't embody the combinations of blue and red lights; however, it's known that this could increase the synthesis of bound compounds, otherwise from the monochromatic lights. The coordination ratio of blue and red LEDs less than one is optimal for anthocyanins accumulation in plants, whereas for flavonoid accumulation, the amount of blue light can be reduced to zero depending on the specific phenolic compounds and specific plant types [24].

A. burmannicus is a shade plant and sensitive to light. However, the consequences of light quality on *A. burmannicus* have not been reported. Therefore, this study hypothesized that *A. burmannicus* plants have a positive effect on the growth and bioactive compounds under LED lights (24 h) compared to natural light (12 h). The purpose of this study was to use energy-saving LED lamps to determine the effects of different light quality on growth, photosynthetic pigments content, antioxidant activity, and accumulation of bioactive compounds in the cultivation stage of *A. burmannicus* and to determine the optimal light quality to increase the medicinal value and economic benefits for the growers.

Materials and methods

The 6 treatments including blue light (BL, 24 h) LED, full spectrum cool light (CL, 24 h), red combine blue LED 1:1 (RBL, 24 h), red light (RL, 24 h), full-spectrum warm light (WL, 24 h) lighting up 24 h per day for 60 days. Natural light (NL, 12 h), provides 12 h (06:00 - 18:00) in a greenhouse and left in darkness,

was used as the control. Morphological growth was measured after 60 days of treatment by counting shoot numbers, stem length, stem diameter, canopy diameter, leaf numbers, leaf width, and leaf length. After that, harvest leaves of *A. burmannicus* were extracted and analyzed photosynthetic pigment contents and bioactive compounds; determine Chlorophyll a, Chlorophyll b, Carotenoid content; Total Polysaccharide; Total Phenolic compared with Gallic acid; Total Flavonoids compared with Rutin trihydrate; antioxidant activity using 2, 2–azino–bis–3–ethylbenzothiazoline–6–sulfonic acid (ABTS) compared with Trolox; antioxidant activity using diphenyl picrylhydrazyl (DPPH) compared with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

Plant materials and growth conditions

Test species: *A. burmannicus*. The experiment was performed during late April - July of 2021 in the Greenhouse system, Biotechnology Program, Faculty of Science, Maejo University, Chiang Mai, Thailand (18.896041, 99.012529) with 20 % sunlight coverup, temperature between 25 - 28 °C, and humidity between 80 - 90 %. *A. burmannicus* plantlets were obtained from a temporary Immersion bioreactor at; Agricultural Research and Extension laboratory and transplanted for acclimatization and cultivated in mixed material soil in 4-inch pots (10.16 cm diameter, 7.62 cm depth) for 9 months before entering the greenhouse. In the greenhouse, these plantlets were transplanted again into the plastic 4-inch pots (10.16 cm diameter, 7.62 cm depth). The cultivation materials were composed of soil: burnt chaff: peat moss: coconut flakes: and perlite in a 1:1:1:1:1 ratio by volume. *A. burmannicus* plantlets were watered slowly until water runs out of the drainage holes, once every 2 days and always fertilized by flake chemical fertilizer orchid, 21−21−21+ TE; 50 g per 20 L of water once a week for 60 days.

Using an LED Lighting technology experiment, LED lamps were provided by A. E. E. GROW LIGHT (Nakhon Pathom, Thailand). Plantlets were exposed to 6 different light quality treatments (**Figure 1)** as follows: cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red: blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h). The spectral characteristics of the lamps, as shown in **Figure 2**, were measured by a PG100N Spectral PAR-PPFD Spectrometer (United Power Research Technology Corporation, Zhunan, Taiwan). Each treatment consisted of 30 pots with 3 replications.

Figure 1 LED lighting shelf in the greenhouse system shows 6 different light quality treatments: blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red: blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h).

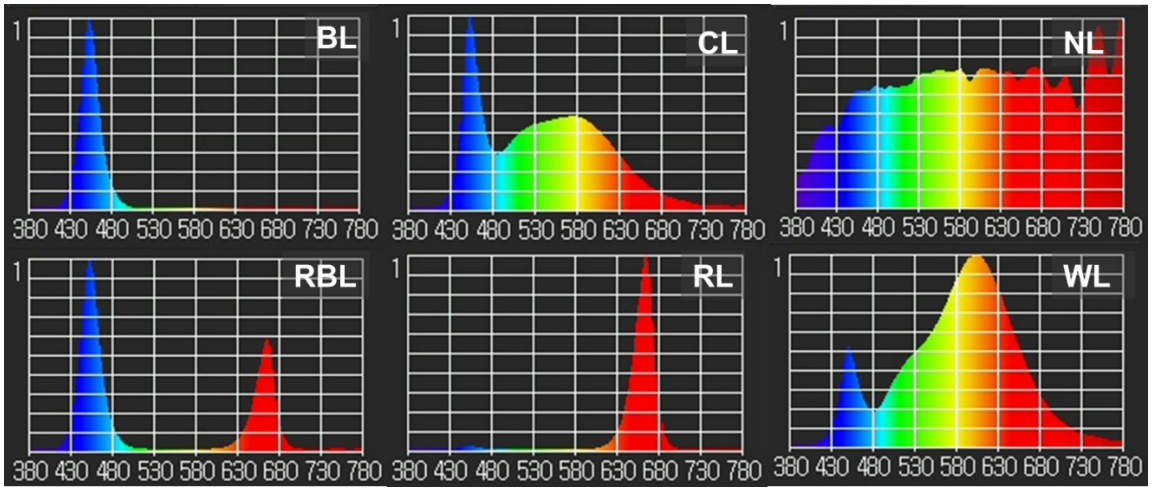

Figure 2 Light spectra of LED lighting with 6 different light quality treatments: blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red: blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h).

The LED lamps were placed above the plantlets. The conclusion from Ma *et al*. [18] was referenced and the height of each light fixture was adjusted to ensure the light intensity was 30 ± 10 m mol m^{-2} s^{-1}. Different treatments were insulated from each other by black shading materials.

Growth and biomass parameter analysis

For measurements of morphological parameters, 15 plants were randomly selected from each treatment, and shoot numbers, stem length, stem diameter, canopy diameter, leaf numbers, leaf width, leaf length, fresh weight, and dry weight were measured. The fresh weight of the plantlets was measured with an electronic balance (Sartorius, Hamburg, Germany), and the plantlets were dried to a stable weight at 70 °C to determine their dry weight. Plant growth parameters were measured with a caliper (Tricle Brand Tools, Shanghai, China).

Photosynthetic pigment contents

Leaf samples were collected for determination of Chlorophyll content (Chlorophyll a and Chlorophyll b) and Carotenoid. The powder samples from each treatment weighed 0.0100 g. and were then extracted with 5 mL. of 80 % Acetone (RCI Labscan, Ireland) in the dark at 4 °C for 24 h. The supernatant was separated, and the absorbance was measured at 440, 647, and 664 nm on a spectrophotometer (Analytik Jena, SPECORD 200 PLUS, Germany). The number of Chlorophyll content was calculated according to the formulars of Porra [25] and expressed mg/g fresh weight as follows:

$$\text{Chlorophyll a} = \frac{(12.25\times\text{optical density}_{664} - 2.55\times\text{optical density}_{647})\times\text{volume of supernatant (mL)}}{\text{sample weight (g)}}$$

$$\text{Chlorophyll b} = \frac{(20.31\times\text{optical density}_{647} - 4.91\times\text{optical density}_{664})\times\text{volume of supernatant (mL)}}{\text{sample weight (g)}}$$

$$\text{Carotenoid} = \frac{[(4.69\times\text{optical density}_{440}\times\text{volume of supernatant (mL)}]}{\text{sample weight (g)}} - [0.267\times (\text{Chl } a + \text{Chl } b)]$$

Bioactive compounds content

This method was modified by Ye *et al.* [26]. Powdered samples (0.5000 g) were accurately weighed and then extracted in a Sonicator with ethanol (RCI LabScan, Ireland) – deionized water (RCI LabScan, Ireland) (80 - 20, v/v) 50 mL at 60 °C for 2 h. The extract solution was filtrated and concentrated with a rotary evaporator (BUCHI, Switzerland).

Total polysaccharide content

The polysaccharide concentration was determined using the phenol-sulfuric acid modified method by Liu *et al.* [27]. The absorbance of the solution was measured at 490 nm, and d-glucose (25 - 400 µg/mL) was used as the standard. The yield of polysaccharides was calculated using the equation: Total polysaccharides (mg/g fresh weight) = [glucose equivalent (mg/mL)×% Yield] / [dry extract in 1 mL (g)× 100].

Total phenolic content

The Folin-Ciocalteu method was used to quantify total phenolics, with gallic acid (0.05 - 1 mg/mL) as the standard. The absorbance of the solution at 760 nm was measured by using a microplate reader. The total phenols were calculated by using the equation: mg GAE/g fresh weight = [equivalent concentration of gallic acid (mg/mL) ×100×% Yield dry×% Yield extract] / [dry extract in 1 mL(mg)×100×100]

Total flavonoids content

The flavonoid content was determined by the $NaNO_2$- Al $(NO_3)_3$- NaOH modified method from Zhu H. *et al.* [28]. Rutin trihydrate (0.008 - 0.5 mg/ml) was used as the standard. The absorbance of the solution was determined by a microplate reader at 510 nm. The yield of flavonoids was calculated using the equation: Total Flavonoids (mg/g fresh weight) = [rutin trihydrate equivalent (mg/mL) ×% Yield dry] / [dry extract in 1 mL(mg) ×100 g.]

Antioxidant activity by ABTS assay

The antioxidant activity was determined by the ABTS radical cation ($ABTS^{\cdot+}$) decolorization assay involving performed ABTS radical cation (modified method from Cao *et al*. [29]). Trolox (0.9 - 120 µg/mL) was used as the standard. The absorbance was then measured at 734 nm by a microplate reader for 30 min. The percentage inhibition was calculated by using the following equation: % inhibition = [(Abs control - Abs sample) / Abs control]. The percentage inhibition of the sample was compared with the standard curve of the Trolox equivalent. The antioxidant activity expressed mg TEAC/g fresh weight.

Antioxidant activity by DPPH assay

The antioxidant activity was analyzed by using the DPPH method adapted from Hatano *et al.* [30]. The mixture of the sample (0.1 - 12 mg/mL) and DPPH reagent (0.2 mM) was shaken vigorously and measured the absorbance at 517 nm for 30 min using a microplate reader. The absorbance of the sample was calculated as Abs sample = A sample - A blank and Abs control = A positive – A negative. The percentage inhibition was calculated by using the following equation: [(Abs control – Abs sample) / Abs control] x 100. Inhibition of antioxidant capacity at 50 % (IC_{50}) was calculated and compared with BHA and BHT as reference standards.

Statistical analysis

IBM SPSS statistics v.28.0.0.0(190) (2021) program was used for all statistical analysis. Data were analyzed by 1 factor, light quality. The results were analyzed by one-way analysis of variance. Duncan's multiple range test was employed to detect differences between means (with p set to 0.05).

Results and discussion

Morphological observations

The growth parameters of *A. burmannicus* treated with different LED light qualities on day 60 are summarized (**Figures 3 - 4** and **Table 1**). The shoot numbers did not differ significantly from all treatments. Significantly higher stem length was observed under the WL treatment (6.09 ± 1.25, $p < 0.05$), at 59.77 % higher than the NL. However, the stem length did not differ significantly from the CL and RL treatments. The canopy diameter was significantly greater in the WL treatment (8.58 ± 1.31, $p < 0.05$) than in the NL. The canopy diameter did not differ significantly from the CL and RL treatments. The greater stem diameter was significant in the WL treatment (3.99 ± 0.43, $p < 0.05$) than in the NL. The stem diameter did not differ significantly from the BL, CL, RBL, and RL treatments. The maximum leaf numbers were significantly in the RBL treatment (6.09 ± 0.63, $p < 0.05$) than in the NL. The leaf numbers did not differ significantly from the CL and RL treatments. The higher leaf length was significantly in the WL treatment (4.23 ± 0.53, $p < 0.05$) than in the NL. The leaf length did not differ significantly from the CL, BL, and RL treatments. The larger leaf width was significant in the CL treatment (2.89 ± 0.36, $p < 0.05$) than in the NL. The leaf width

did not differ significantly from the BL, and RBL treatments. Fresh weight (24.16 ± 8.81 g, $p < 0.05$) and dry weight (2.74 ± 0.98 g, $p < 0.05$) of the BL treatment were significantly greater than the other treatments.

Figure 3 Appearance of *A. burmannicus* grown under 6 different light quality treatments: blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red: blue 1: 1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h) for 60 days.

Table 1 Effects of 6 different light quality treatments on morphology parameters and biomass of *A. burmannicus*.

Treatment	BL	CL	NL	RBL	RL	WL
Shoot numbers	1.17 ± 0.40 ^{a}	1.17 ± 0.42 ^{a}	1.18 ± 0.45 ^{a}	1.20 ± 0.45 ^{a}	1.19 ± 0.39 ^{a}	1.13 ± 0.37 ^{a}
Stem length (cm)	5.72 ± 1.07 ^{b}	4.90 ± 1.23 ^{c}	3.64 ± 1.05 ^{d}	5.89 ± 1.11 ^{ab}	4.94 ± 1.14 ^{c}	6.09 ± 1.25 ^{a}
Stem diameter (cm)	3.86 ± 0.49 ^{ab}	3.73 ± 0.48 ^{b}	3.45 ± 0.45 ^{c}	3.79 ± 0.38 ^{b}	3.73 ± 0.43 ^{bc}	3.99 ± 0.43 ^{ab}
Canopy diameter (mm)	8.08 ± 1.06 ^{a}	7.50 ± 1.54 ^{b}	6.80 ± 1.00 ^{c}	8.32 ± 1.12 ^{a}	7.40 ± 1.32 ^{bc}	8.58 ± 1.31 ^{a}
Leaf numbers	6.00 ± 0.72 ^{a}	5.51 ± 1.04 ^{b}	3.83 ± 0.83 ^{c}	6.09 ± 0.63 ^{a}	5.57 ± 0.87 ^{b}	5.92 ± 0.78 ^{a}
Leaf width (cm)	2.71 ± 0.55 ^{bc}	2.89 ± 0.36 ^{a}	2.44 ± 0.28 ^{d}	2.78 ± 0.40 ^{bc}	2.64 ± 0.30 ^{cd}	2.84 ± 0.32 ^{ab}
Leaf length (cm)	3.95 ± 0.68 ^{b}	3.88 ± 0.57 ^{b}	3.51 ± 0.50 ^{c}	4.08 ± 0.49 ^{ab}	3.92 ± 0.47 ^{b}	4.23 ± 0.53 ^{a}
Fresh weight (g)	24.16 ± 8.81 ^{a}	20.22 ± 6.09 ^{ab}	10.47 ± 0.54 ^{b}	21.02 ± 9.83 ^{ab}	16.69 ± 4.59 ^{ab}	21.1 ± 3.38 ^{ab}
Dry weight (g)	2.74 ± 0.98 ^{a}	2.24 ± 0.68 ^{ab}	1.15 ± 0.07 ^{b}	2.40 ± 1.07 ^{ab}	1.75 ± 0.41 ^{ab}	2.29 ± 0.34 ^{ab}

Notes: Values represent mean ± SE of fifteen replicates, and different letters within a row indicate significant differences at $p < 0.05$.

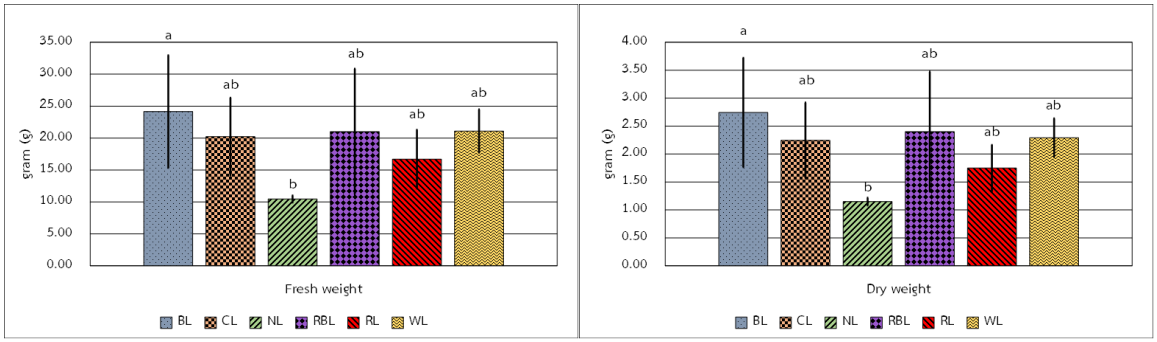

Figure 4 Fresh weight and dry weight of *A. burmannicus* grown under 6 different light quality treatments: Blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red:blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h) for 60 days.

Light plays an important role in plant growth and the accumulation of secondary metabolites. In the present study, the morphological parameters of *A. burmannicus* differed in their responses to different LED light qualities and natural light. It was shown that *A. burmannicus* had significantly better growth responses to LED light exposure (24 h) in all treatments than the natural light (12 h) without any photoperiod related injury. Our result generally agreed with the other reports that long photoperiods of lighting, continuous (24 h) have the potential for increased yield [13-16].

In different LED light qualities, plants of *A. burmannicus* grown under the full spectrum, WL had significantly increased stem length, canopy diameter, leaf numbers, and leaf length. The BL treatments significantly increased the fresh weight and dry weight of *A. burmannicus*. There were similar conclusions in other related studies involving, *Stevia rebaudiana* Bertoni, *Cardamine fauriei,* and *Rehmannia glutinosa* [31-33]. *A. roxburghii*, where exposure to the BL treatment showed the largest leaf numbers [23]. The BL is more effective in enhancing fresh weight and dry matter production in buckwheat sprouts [34] and *A. roxburghii* [23]. These results indicate that plant species differ in their responses to light quality, but the BL treatment generally promotes plant growth and biomass accumulation.

Photosynthetic pigment content

Different LED light qualities had variable effects on the chlorophyll content of *A. burmannicus* (**Figure 5**). Significantly higher Chlorophyll a content (74.89 ± 0.12 mg g^{-1} FW, $p < 0.05$) and Chlorophyll b content (17.52 ± 0.11 mg g^{-1} FW, $p < 0.05$) were observed with the NL treatment than the other treatments. The Chlorophyll b content did not differ significantly from the BL and WL treatments. The significantly higher Carotenoid content (39.00 ± 0.18 mg g^{-1} FW, $p < 0.05$) was in the RBL treatment. The Carotenoid content did not differ significantly from the NL and WL treatments.

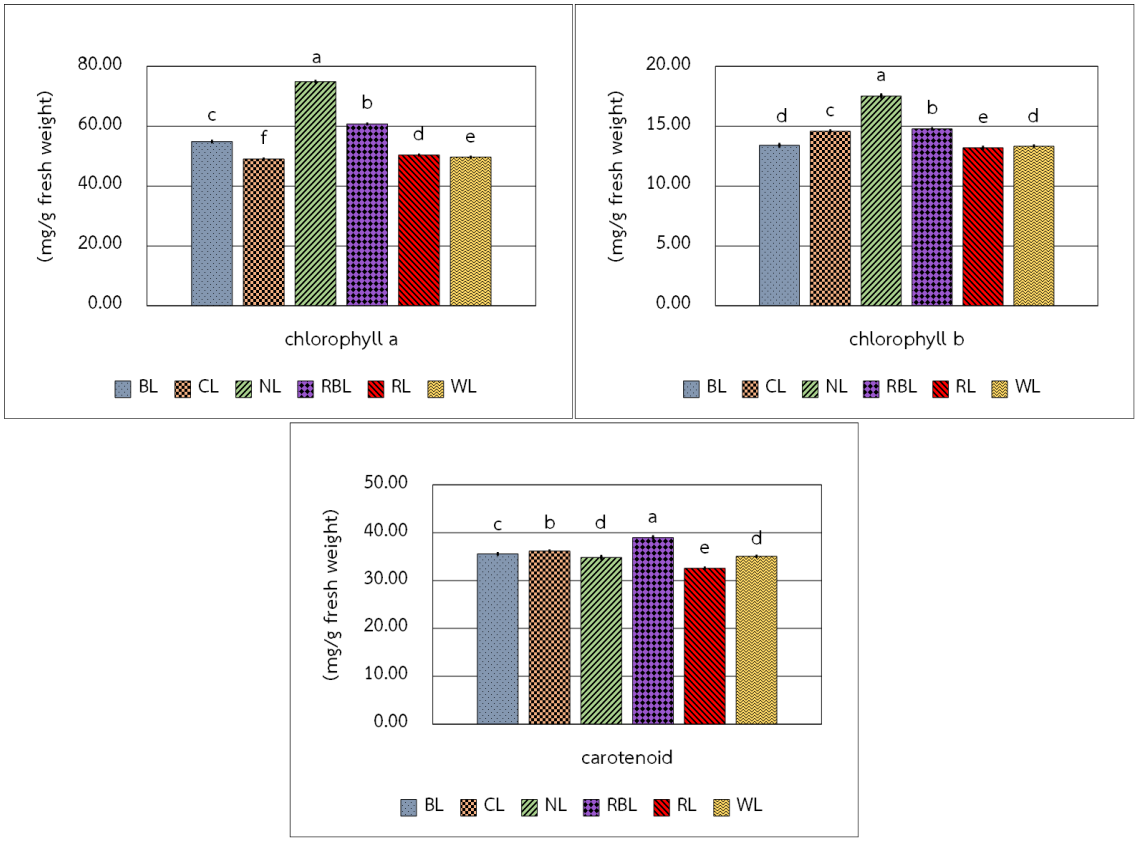

Figure 5 Photosynthetic pigments content of *A. burmannicus* grown under 6 different light quality treatments: Blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red:blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h) for 60 days.

Chlorophylls and Carotenoids are molecules that play an important role in the photosynthetic pathway; the content of these pigments in plants responds to the spectrum of light and intensity to such an extent that their content can be increased or decreased by subtle differences in the light [10]. In this study, the NL treatment had the strongest effect to stimulate Chlorophyll a and Chlorophyll b accumulation and the higher Carotenoid accumulation has been shown to increase in the RBL treatment. The higher Chlorophyll content in the NL treatment than the other treatments illustrated that *A. burmannicus* can maximize its light-harvesting capacity under natural light treatments more than LED light treatment. These findings were supported by Bautista *et al.* [35] that among different lights tested, growing plants using different lighting systems in an experimental study, the plant under sunlight garnered the highest and most consistent observational value for positive features followed by the artificial light LED. Plants modify their Chlorophyll content with the light spectrum. The confliction effects of red and blue light on the pigment contents have been reported in the literature. This indicates that plant responses are very different among species. Monochromatic light (blue or red) has generally been shown to decrease the chlorophyll content in plants [12]. In addition, some authors reported spinach, radishes, lettuce, and einkorn that, when blue light is present with other wavelengths, the Chlorophyll content tends to increase with the given amount of blue light [36].

Bioactive compounds content

The total polysaccharides content in *A. burmannicus* differed among the treatments and ranged from 6.45 ± 0.10 to 17.15 ± 0.16 mg g^{-1} FW. The significantly higher total polysaccharide content was in the BL treatment (17.15 ± 0.16 mg g^{-1} FW, $p < 0.05$), at 265.89 % higher than the NL treatment (6.45 ± 0.10 mg g^{-1} FW, $p < 0.05$). The total phenolics content was significantly higher in the BL treatment (1.10 ± 0.32 mg g^{-1} FW, $p < 0.05$), at 220 % higher than the NL treatment (0.50 ± 0.14 mg g^{-1} FW). The total flavonoid content ranged from 0.47 ± 0.21 (BL) to 1.00 ± 0.17 mg g^{-1} FW (RL). The total flavonoid content was significantly higher in the RL treatment (1.00 ± 0.17 mg g^{-1} FW, $p < 0.05$), at 144.92 % higher than the NL

treatment (0.69 ± 0.16. mg g^{-1} FW). The total flavonoid content did not differ significantly from the BL, CL, NL, and WL treatments. The antioxidant activity of ABTS was significantly higher in the RBL treatment (2.026 ± 0.038 mg TEAC g^{-1} FW, $p < 0.05$), at 137.35 % higher than in the NL treatment (1.475 ± 0.017 mg TEAC g^{-1} FW). The antioxidant activity of ABTS did not differ significantly from the BL and CL treatments. However, the antioxidant activity of DPPH did not differ significantly from all treatments (**Figure 6**).

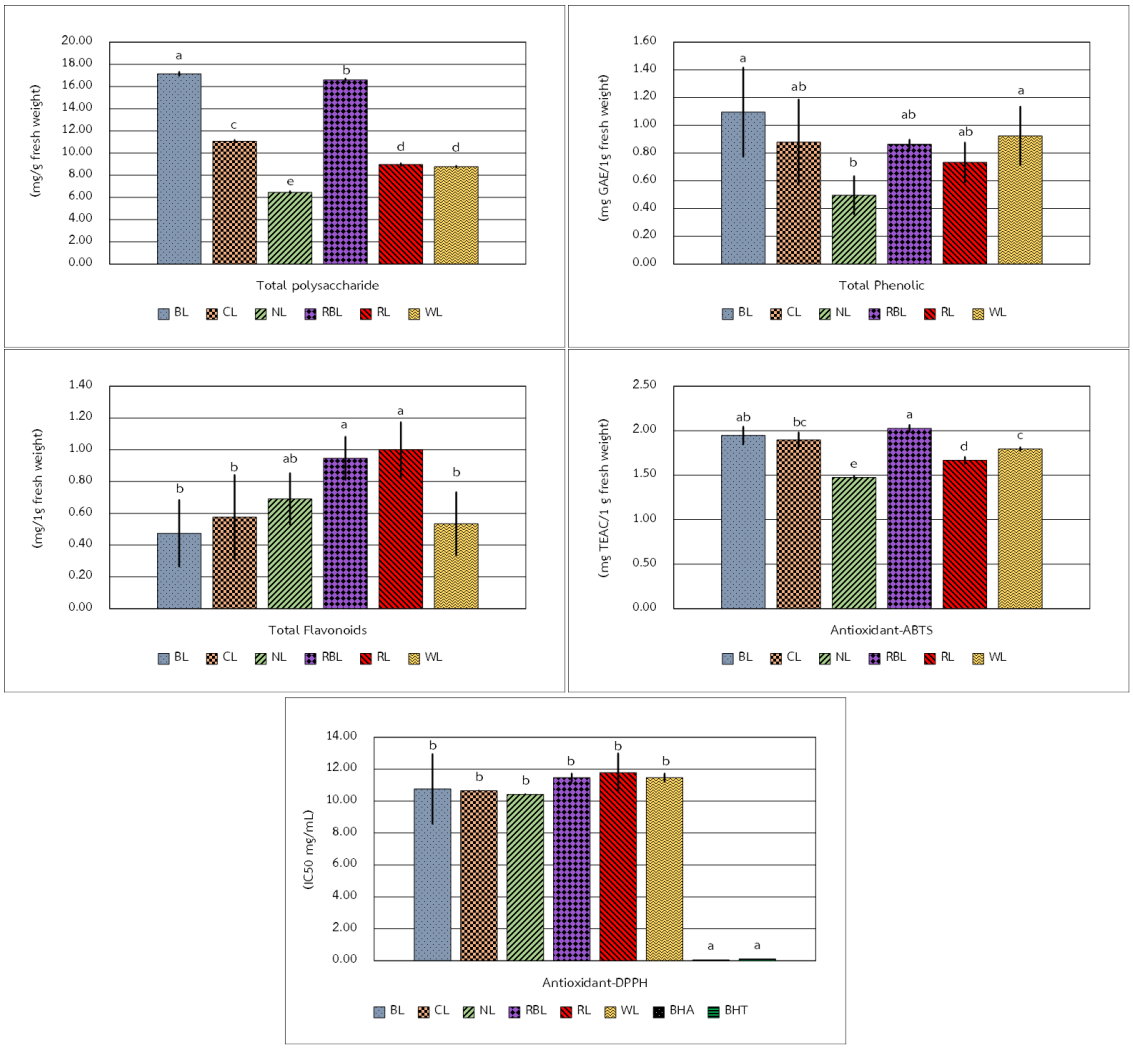

Figure 6 Bioactive compounds content of *A. burmannicus* grown under 6 different light quality treatments: Blue light treatment (BL, 24 h), cool light treatment (CL, 24 h), natural light treatment (NL, 12 h), red:blue 1:1 light treatment (RBL, 24 h), red light treatment (RL, 24 h), and warm light treatment (WL, 24 h) for 60 days.

Secondary metabolism is a necessary part of plant life and closely relates to plant growth and environmental factors. Light is an important environmental factor affecting the accumulation of secondary metabolites. Blue light promotes the accumulation of polysaccharides. This is achieved by increasing the Ca^{2+}- CaM signal's control of the photosynthetic apparatus or glucose metabolism [37]. In *Dendrobium catenatum*, red light was shown to promote the accumulation of carbohydrates, thereby increasing the polysaccharide content [38]. In contrast, blue light promoted polysaccharide accumulation in *Astragalus membranaceus*, to a level 23.9 % higher than that in the control [39]. Our results were consistent with that finding, as BL treatment had the strongest stimulatory effect on total polysaccharide accumulation in *A. burmannicus* among all of the treatments.

Light promotes the accumulation of phenolic compounds, via increased production of malonyl CoA and coumaroyl CoA that serve as substrates for phenolics biosynthesis [40]. Johkan *et al*. [41] reported that blue light promoted the accumulation of phenolic compounds in *Lactuca sativa* seedlings. In sweet

basil, the total phenolics content was lower in a blue light treatment than in a white light treatment [42]. In our study, significantly higher total phenolic content in *A. burmannicus* was observed in the BL treatment.

In *Arabidopsis thaliana*, the blue light receptor cryptochromes (CRY1 and CRY2) and PhyA mediate responses to blue light to promote flavonoid biosynthesis and accumulation [43]. In *A. roxburghii*, the flavonoid content was higher in the BF treatment than in the other treatments [26]. In the present study, the total flavonoid content in *A. burmannicus* was higher under the RL and RBL treatments than in the BL treatments.

Secondary metabolite synthesis in plants; phenol and flavonoid compounds are an important part of the defense response to stress. Oxidative stress and reactive oxygen species (ROS) cause great damage to plants. Žukauskas *et al.* [44] showed that complementary red light had an increasing effect on DPPH removal ability in lettuce, but the antioxidant ability of rice leaves in response to blue LED light was higher than that in red LED light [45]. Ahmadi *et al.* [46] reported that red combination with blue LED lights and then red LED had the greatest effect on increasing radical scavenging activity (RSA) in plants of 2 genotypes of lemon balm (*Melissa officinalis*), particularly in the Ilam genotype. The antioxidant activity in *A. burmannicus*, ABTS was significantly higher in the RBL treatment, while the antioxidant activity of DPPH did not differ significantly between all treatments.

Conclusions

In the present study, *A. burmannicus* plants have a positive effect on the growth and bioactive compounds under LED lights (24 h) compared to natural light (12 h). It can be concluded that BL treatment is required for the normal growth, biomass, and accumulation of polysaccharide and phenolic of *A. burmannicus*, while RBL is enhanced the accumulation of flavonoid and antioxidant activity. Further research will focus on the combination of RBL, especially effective on the bioactive compound.

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