

Enhanced biomass and biological activity of 'Hasuo' (*Polygonum multiflorum* Thunberg) grown under LED light

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Abstract

Polygonum multiflorum is a medicinal plant with strong antioxidant, anti-inflammatory, and cholesterol-lowering effects. We sought to improve the effectiveness of its agronomic characteristics and biological activities through exposure to light-emitting diode (LED) light. Seed germination was increased by $17.3 \pm 4.7\%$ in LED light-treated seeds of *P. multiflorum* compared with fluorescent light-treated (control) seeds ($13 \pm 4.2\%$). The biomass of the aerial parts of *P. multiflorum* increased to the greatest degree, with a stem length of 65.6 ± 4.2 cm and a leaf length of 5.4 ± 1.3 cm following red LED light treatment compared with control plants (stem length, 14.7 ± 4.5 cm; leaf length, 4.6 ± 1.2 cm). The epidermal cell size was $76.5\text{--}87.9$ μm following exposure to red LED light by scanning electron microscopy based on biomass data. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity, total phenolic content, and total flavonoid content, including antioxidant activity, were also evaluated. In terms of DPPH activity, the samples extracted from roots had the highest reducible concentration 50% (RC_{50} ; 151.38 ± 0.31 $\mu\text{g ml}^{-1}$) following blue LED light treatment. The total phenolic content was highest (535.07 $\mu\text{g GAE ml}^{-1}$) in roots following blue LED light treatment. The total flavonoid content was highest (251.23 $\mu\text{g QE ml}^{-1}$) in leaves following blue LED light treatment. Physiological analyses indicated increases in the biomass and biological activity of *P. multiflorum* treated with LED light. Our results suggest that treating *P. multiflorum* with LED light for 5 weeks can enhance food manufacturing and pharmaceutical production from natural substances.

Keywords: Biomass, DPPH, LED, *Polygonum multiflorum*, SEM.

Abbreviations: DPPH_2,2-diphenyl-1-picrylhydrazyl; GR_germination rate; LED_light-emitting diode; RC_{50} _reducible concentration 50%; ROS_reactive oxygen species; SEM_scanning electron microscopy.

Introduction

Polygonum multiflorum Thunberg is a popular herbal medicine marketed as 'He Shou Wu' in East Asia and North America (Shahidi et al., 1992). It is used as an anti-aging treatment and for the blackening of hair displaying early graying (Chan et al., 2003). Its roots possess various biological properties, including anti-tumor and antibacterial activities (Buyukokuroglu et al., 2001). It contains phenolic compounds, including anthraquinones, stilbenes, and tannins. A major constituent of *P. multiflorum*, stilbene glycoside, has been shown to have neuroprotective effects against ischemia/reperfusion injury (Wang et al., 2009; Ye et al., 2006). The pharmacological effects of *P. multiflorum* have also been reported, including blood cholesterol-lowering, anti-oxidative, and anti-inflammatory effects (Liu et al., 2011; Wang et al., 2007; Yang et al., 2005; Zhang et al., 2007). Several environmental factors affect plant growth, including temperature, light intensity, air movement, and humidity. Of these, light is a major factor that directly or indirectly influences the growth and morphology of plants and regulates the size of stems and leaves (Debergh et al., 1992). Light is an important signal that influences development and gene expression during the life cycle of plants (Erdei et al.,

2005). As an artificial light source, light-emitting diodes (LEDs) can be used to control the environment for plant growth (Okamoto et al., 1997). The radiation from LED lights influences lettuce, pepper, spinach, and wheat crop breeding (Wu et al., 2007). The red light from LED lamps is known to influence photosynthesis and starch accumulation in plant leaves (Sae bo et al., 1995). Blue LED light is known to promote the opening of stomata, the formation of chlorophyll, and the development of chloroplasts in plants (Wu et al., 2007). The medicinal traits of plants have recently been highlighted by several scientific developments. Free radicals cause oxidative damage that can be prevented by stopping the oxidation process and by the action of oxygen scavengers (Patel et al., 2010). Reactive oxygen species (ROS) are created in the body, and can be broken down by antioxidants (Patel et al. 2010). Butylated hydroxytoluene, butylated hydroxyanisole, and tertiary butyl hydroquinone are standard chemicals that possess antioxidant activity (Moure et al., 2001). There is demand for antioxidants from natural sources that are safe for use in humans (Rimbach et al., 2005). Several novel antioxidative phenolic compounds, including flavonoids, have been isolated from plants (Tada et al., 1996; Chuda et al., 1996). Flavonoids have been identified

as strong antioxidants, and a relationship between antioxidative activity and the phenolic content of plants has been reported (Azuma et al., 1999). Furthermore, flavonoids and phenolic compounds from crude extracts of many plants have been shown to exert multiple biological effects (Miller, 1996). Phenolics are popular in the food industry because they can retard the oxidative degradation of lipids, while flavonoids exhibit free radical scavenging and anti-inflammatory activities (Frankel, 1995). In this study, we examined how LED light affects the growth and morphology of plants. We also evaluated the antioxidant capacity (by measuring 2,2-diphenyl-1-picrylhydrazyl [DPPH] activity) and total phenolic and flavonoid contents of *P. multiflorum* Thunberg exposed or not exposed to LED light. We also investigated the growth of the root, stem, and leaves of *P. multiflorum* Thunberg.

Results

Germination of P. multiflorum treated with LED light

Germination started on the third day after sowing under fluorescent (control) and red LED light but not under blue LED light or dark treatment. The germination rate (GR) after 10 days was calculated (Fig. 1). The highest GR in *P. multiflorum* was $17.3 \pm 4.7\%$ following blue LED light treatment compared with fluorescent light treatment ($13 \pm 4.2\%$), whereas the lowest GR ($10.0 \pm 1.0\%$) occurred under dark treatment (Fig. 1).

Polygonum multiflorum biomass under LED light treatment

The germination of *P. multiflorum* seeds was evaluated under control, blue LED light, red LED light, and dark conditions. Anthocyanins accumulated in the red part of the hypocotyl in plants grown under blue LED light (Fig. 2). The characteristics of the germinated seeds were compared among plants grown under fluorescent, blue, and red LED lights for 5 weeks. Greater aboveground growth was detected under red LED lights than under the other lights; these plants developed more fine roots compared to the controls (Fig. 2). Further, the roots were longer and thicker than in the plants exposed to blue LED light (Fig. 2), and the back of each leaf and stem became red-colored and accumulated anthocyanin (Fig. 3). The biomasses of the aboveground and belowground parts of the plants were also determined (Table 1). The greatest plant height (65.5 cm), leaf length (5.4 cm), and leaf width (3.3 cm) were observed in plants grown under red LED light. In addition, a 1.8-fold increase in dry weight (6.5 g) compared to the control plants was observed. The lowest plant height (14.7 cm), leaf length (4.6 cm), and leaf width (3.0 cm) were recorded in the controls. The longest root length (5.8 cm) was observed under blue LED light, but the root diameter was greatest (7.4 mm) under red LED light. The root dry weights were 7.21 g under blue LED light and 11.79 g under red LED light, compared to 0.6 g in the controls (11- and 18-fold increases, respectively; Table 1).

Morphological characterization by scanning electron microscopy (SEM)

SEM was used to evaluate morphological differences (Fig. 3). The upper epidermal cells of the *P. multiflorum* leaves protruded upwards following exposure to blue LED light, whereas SEM indicated that the cells were turgid. The epidermal cells were smallest ($29.5\text{--}46.2\text{ }\mu\text{m}$) under blue LED light and largest ($76.5\text{--}87.9\text{ }\mu\text{m}$) under red LED light compared with the controls. By SEM, the cells around pores in the stems

were found to have many wrinkles under red LED light, but no major differences between blue LED and fluorescent light treatment were identified. SEM of stem cross-sections revealed that the area near the cambium contained a slightly thickened formation resulting from the accumulation of substances under blue and red LED lights, but there was no significant difference compared to the controls (Fig. 3).

DPPH free radical scavenging activity

We determined the reducible concentration 50% (RC_{50}) value ($\mu\text{g/ml}$) for the antioxidant activity of methanol extracts of various plant parts using a DPPH free radical scavenging assay. The antioxidant activity was higher ($151.38 \pm 0.31\text{ }\mu\text{g/ml}$) in the root extracts compared to the stem and leaf extracts (Table 2). The methanolic extract of stems treated with blue LED light had low DPPH scavenging activity ($535.32 \pm 0.33\text{ }\mu\text{g/ml}$), whereas the control stem extract exhibited the lowest value ($223.41 \pm 0.32\text{ }\mu\text{g/ml}$) (Table 2). Leaves grown under blue LED light had a lower RC_{50} ($243.52 \pm 0.30\text{ }\mu\text{g/ml}$) than control leaves ($303.64 \pm 0.29\text{ }\mu\text{g/ml}$). The antioxidant activity of *P. multiflorum* grown under LED light was twice that of the control plants.

Total phenolic content according to LED light exposure

A standard calibration curve for total phenolics was generated using gallic acid ($R^2 = 0.9965$). The root methanolic extract of *P. multiflorum* grown under blue LED light had the highest total phenolic content ($535.07\text{ }\mu\text{g GAE/ml}$). The total phenolic content was lowest ($352.03\text{ }\mu\text{g GAE/ml}$) in the root extract of plants grown under red LED light (Fig. 4). The total phenolic contents of the stem and leaves of plants grown under both LED light treatments did not differ significantly compared to the controls (Fig. 4).

Total flavonoid content according to LED light exposure

We investigated the flavonoid content of *P. multiflorum* compared to a quercetin standard ($R^2 = 0.9989$). The highest total flavonoid content was in leaf extracts ($251.23\text{ }\mu\text{g QE/ml}$) of *P. multiflorum* grown under blue LED light (Fig. 5). Samples from stems or roots grown with/without LED light treatment did not differ in total flavonoid content (Fig. 5). Substantial total flavonoid contents were identified by DPPH free radical scavenging and total phenol assays in plants grown under blue LED light.

Discussion

A number of factors influence seed germination. Internal factors impact immature seeds regardless of air or moisture, and external factors include oxygen, temperature, moisture, and light (Bewley and Black, 1982; Dennis, 1995). Light is known to influence seed germination (Kwack and Kang, 1985). The GR of rapeseed is highest (92%) under red LED light and lowest (74%) under blue LED light (Heo et al., 2008). This conflicts with our finding that blue LED light treatment was more suitable for the germination of *P. multiflorum*. *Polygonum multiflorum* is thought to be a light germinator, as shown by its higher germination ratio under white fluorescent light, red LED light, and blue LED light compared to in the dark. In the future, a mixture of blue and red LED light should be used to determine

Table 1. Biomass of aerial and underground parts of *Polygonum multiflorum* under the different light emitting diode (LED) color conditions.

Light source	Aerial part				Underground part		
	Stem length (cm)	Leaf length (cm)	Leaf width (cm)	Dry weight (g)	Root length (cm)	Root diameter (mm)	Dry weight (g)
Control	14.7±4.5c	4.6±1.2c	3.0±1.1c	3.6±1.0c	4.6±1.3b	2.1±0.8c	0.6±0.3c
Blue	32.8±3.7b	4.8±1.5b	3.1±0.9b	5.3±0.9b	5.8±2.1a	4.9±1.4b	7.2±1.7b
Red	65.5±4.2a	5.4±1.3a	3.3±1.3a	6.5±1.4a	2.8±1.8c	7.4±1.9a	11.8±1.6a

Within columns, means followed by the same letter did not differ significantly according to Tukey's honestly significant difference test ($P < 0.05$).

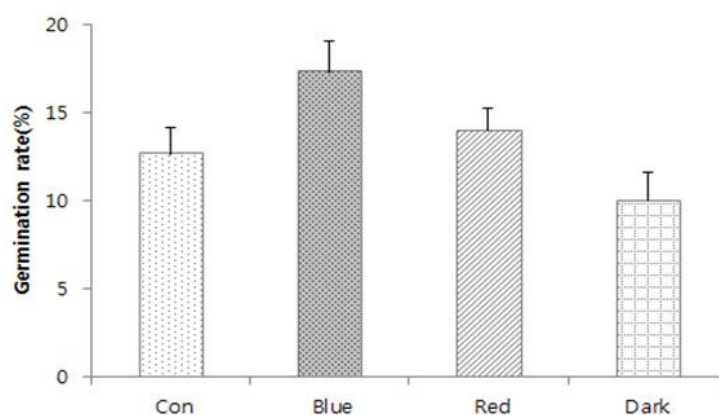


Fig 1. Germination effect of *Polygonum multiflorum* seed in condition of LED lights for 10 days. Within columns, means followed by the same letter did not differ significantly according to Tukey's honestly significant difference test ($P < 0.05$).

Table 2. DPPH¹⁾ free radical scavenging activities of aerial part and underground part of *Polygonum multiflorum* with and without LED treatments.

Plant parts	LED Light sources	RC ₅₀ ²⁾ (μg/ml)
Root	Control	273.37 ± 0.29e
	Blue	151.38 ± 0.31i
	Red	166.60 ± 0.32h
Leaf	Control	303.64 ± 0.29d
	Blue	243.52 ± 0.30f
	Red	454.75 ± 0.27b
Stem	Control	223.41 ± 0.32g
	Blue	535.32 ± 0.33a
	Red	451.41 ± 0.32c
Ascorbic acid		5.74 ± 0.14k
BHT		71.52 ± 0.24j

¹⁾ DPPH : 1,1-diphenyl-2-picrylhydrazyl; ²⁾ RC₅₀ : Amount required for 50% reduction of DPPH after 30 min. Each value is mean ± standard derivation of three replicate tests. Within columns, means followed by the same letter did not differ significantly according to Tukey's honestly significant difference test ($P < 0.05$).



Fig 2. Difference of morphology and growth of *Polygonum multiflorum* under the different light emitting diode (LED) color conditions. Con; control, Blue; LED blue light treatment and Red; LED red light treatment.

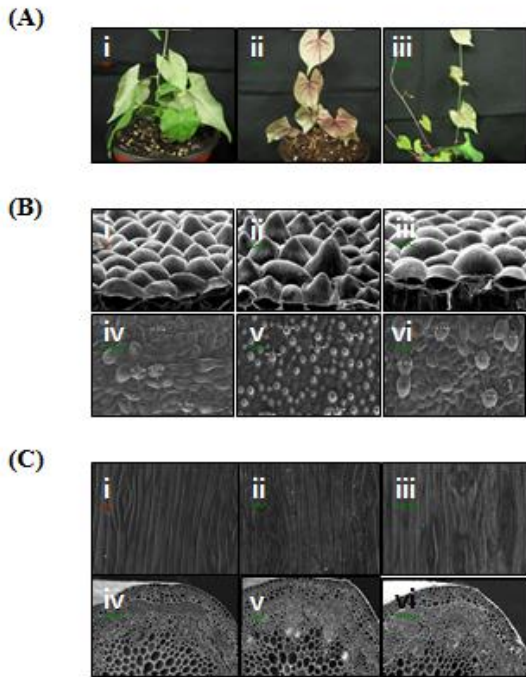


Fig 3. Morphological difference in phenotype and SEM analysis of *Polygonum multiflorum* grown under the different light emitting diode (LED) color conditions. (A) i: Control; ii: Blue light; iii: Red light. (B) Epidermal cell of *Polygonum multiflorum* upper leaf surface by SEM analysis. i, iv: Control; ii, v: Blue light; iii, vi: Red light. (C) Stoma and cross section of stem by SEM analysis. i, iv: Control; ii, v: Blue light; iii, vi: Red light.

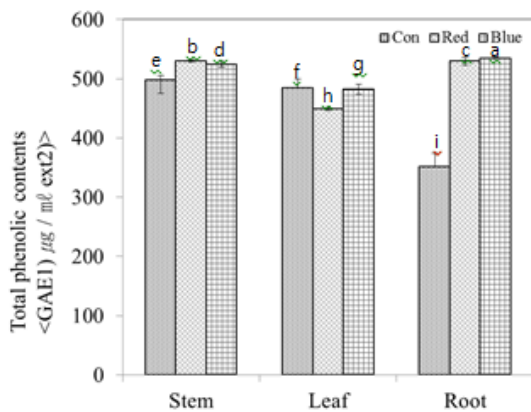


Fig 4. Total phenolic contents from extracts of *Polygonum multiflorum* grown under different light emitting diode (LED) color conditions. Within columns, means followed by the same letter did not differ significantly according to Tukey's honestly significant difference test ($P < 0.05$).

if the mean number of days required for germination can be reduced and the germination ratio can be increased. Anthocyanins accumulated on the backs of the leaves and stems, which developed a red color under blue LED light (Fig. 3). Light quality is thought to act as a regulatory factor, as well as being the source of energy for growth, morphogenesis, and pigment production in plants. Anthocyanins are expressed under blue and ultraviolet (UV)-A light, and cryptochromes act

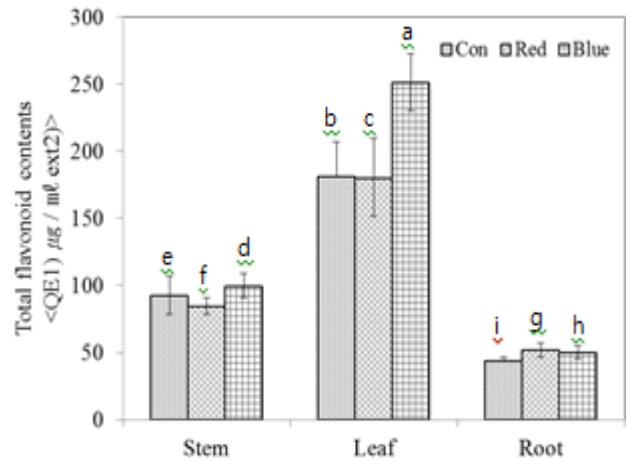


Fig 5. Total flavonoid contents from extracts of *Polygonum multiflorum* grown under different light emitting diode (LED) color conditions. Within columns, means followed by the same letter did not differ significantly according to Tukey's honestly significant difference test ($P < 0.05$).

as blue light receptors (Fankhauser and Chory, 1997; Giliberto et al., 2005). Blue light has been reported to control anthocyanin biosynthesis by facilitating the expression of chalcone synthase and dihydroflavonol-4-reductase (Meng et al., 2004). Regarding biomass, the plant height, leaf length and width, and dry weight were greatest under red LED light. Similar results have been reported for sesame (i.e., the plant height increased by 7.8 cm under red LED light compared with blue light, and the root length was greater) (Choi, 2003). We thus suggest that light quality could be used to control crop cultivation and utilization. LED lights can be used to promote vegetative growth, plant size, leaf growth, and stem morphology because leaves readily absorb blue and red light (Wu et al., 2007). The stem length, leaf area, and seedling weight of potato plantlets is regulated by the quality of light radiation (Miyashita et al., 1995). The growth of vegetables can be changed following exposure to LED light, and the photomorphogenic pigments produced are implicated in regeneration and photoperception (Lian et al., 2002). The backs of the leaves and stems exhibited anthocyanin accumulation during growth and morphogenesis, depending on the quality of the blue LED light (Fig. 3). Light quality is an external environmental factor affecting plant shoot height, leaf shape, growth, morphogenesis, and chlorophyll synthesis (Wongnok et al., 2008). SEM revealed no marked difference in the pores of the stems between control plants and those grown under blue LED light (Fig. 3). The upper epidermal cells on the leaves of plants grown under red LED light billowed outward (Fig. 3). This morphology was likely caused by anthocyanin accumulation. Plants treated with blue LED light produce sufficient amounts of anthocyanin to offer protection from environmental changes (Tattini et al., 2004). A range of blue light wavelengths triggers responses to environmental signals similar to those induced by UV light (Ebisawa et al., 2008). These morphological variations likely provide plants exposed to blue LED light with increased resistance to environmental changes, depending on the level of anthocyanin accumulation. DPPH is a purple compound composed of stable free radical molecules with a specific photosynthetic absorbance at 517 nm that can be readily stabilized in a general organic solvent for use as a substrate in antioxidant assays

(Oyaizu, 1986). Its activity is based on electron donation by a proton radical scavenger in various antioxidative mechanisms (Choi and Oh, 1985). Similar to the findings of this study, DPPH radical scavenging activity was increased by 90.0 and 90.3% in rape under LED light (Cho et al., 2008). This is likely due to the impact on antioxidant activity (i.e., promotion of anthocyanin synthesis by blue LED light). Therefore, LED light can be regarded as useful because antioxidant activity is believed to be the major human health benefit of bioactive compounds (Table 2). Phenolic compounds are secondary metabolites of plants that are involved in a diverse range of biological activities. The antioxidant activity of lettuce was increased significantly following blue light treatment, as indicated by the total phenolic content (Kook et al., 2013). The growth and anthocyanin content of dandelion were enhanced following treatment with LED light, compared with other light treatments (Ryu et al., 2012). Choi et al. (2004) reported a close relationship between total phenol content and antioxidant activity. Plant-derived phenolic compounds can inhibit oxidation and exert antiallergic, antifungal, and anticancer effects (Azuma et al., 1999). The samples extracted from roots had a higher total phenol content under blue LED light (Fig. 4). Therefore, extracts of *P. multiflorum* can influence antioxidative activity. Flavonoids, which are present in many plants, including vegetables and fruits, exert anti-allergy, anti-hypertension, and anti-inflammatory effects (Hong, 2009). Because most plants have a high phenol content, the flavonoid content is also high (Choi et al., 2005). The flavonoid content of plants is thought to be associated with the antioxidant activity (Das and Pereira, 1990). Flavonoids are present in more than 4000 species and have considerable antioxidative, anti-inflammatory, anti-fungal, and capillary action properties (Cha and Cho, 2001). The total flavonoid content was higher in the extracts of plants treated with blue LED light (Fig. 5). These results can be applied to the production of functional foods by increasing the activities of the aerial and underground parts of *P. multiflorum* plants exposed to blue LED light. Red LED light could be used on farms that require high crop growth rates and yields. Further research into blue and red LED light will enable their beneficial effects on the biological activity and yield of crops to be applied to diverse industries.

Materials and Methods

Preparation of seed material

Seeds of *P. multiflorum* were obtained from the Chungbuk Agricultural Research Center. Seed germination tests were performed under various growth conditions by culturing in pots containing nursery soil at room temperature.

Germination of *P. multiflorum* seeds under LED light

A seed germination test was performed using the top-of-paper method. *Polygonum multiflorum* seeds were used after being selected for size and weight following storage at 5°C in a refrigerator. Thirty seeds were inoculated into 90 × 15-mm disposable Petri dishes into which two filter papers had been placed. This was repeated three times. We then performed a germination test using a fluorescent lamp (control light treatment, 1700 lux), blue LED light (465 nm, 520 lux), and red LED light (630 nm, 2050 lux) at 25°C in a plant tissue culture room (16 h light/8 h dark). Distilled water was applied once per day to keep the seeds from drying out. We examined the degree of germination daily for 10 days after sowing.

The GR was calculated by considering seeds with radicles longer than 2 mm to have germinated.

Biomass of plants grown under LED light

The germinated seeds were moved to pots containing autoclaved soil. Five samples were transferred to a plant tissue culture room at 25°C and exposed to fluorescent light, blue LED light, or red LED light treatment for 5 weeks. They were harvested periodically, and the plant height, leaf length, and leaf width of the aboveground parts were measured. Additionally, the root length, root weight, and dry weight of the belowground parts were determined using a measurement tool and Vernier calipers (CD-15CPX; Mitutoyo, Kawasaki, Japan).

Morphological characterization

Stems and leaves of *P. multiflorum* were collected and used to examine plant growth under the various light treatments over a 5-week period. The leaves and stems were washed with phosphate buffer at 4°C and then reacted with 3% glutaraldehyde and phosphate buffer for 5 min. The samples were immersed in 3% glutaraldehyde and phosphate buffer (4°C) for 2 h. The fixed samples were dipped into phosphate buffer (4°C) for 2 h and then washed. They were then fixed again in 1% O₆, O₄ phosphate buffer at 4°C. The samples were then dehydrated twice for 5 min in 50% acetone, twice for 5 min in 70% acetone, twice for 10 min in 80% acetone, twice for 15 min in 90% acetone, and twice for 20 min in 100% acetone and then dried. Finally, the samples were fixed as a paste, coated with gold, and visualized by LV-SEM (S-3500N; Hitachi, Tokyo, Japan) at the Korea Basic Science Institute (Chuncheon Center, Daejeon, Korea).

Preparation of plant samples

Crude root, stem, and leaf extracts of *P. multiflorum* were prepared for antioxidant assays. The plants were supplied by Chungbuk Agricultural Research Center. The samples (300 g each) were ground using a homogenizer and extracted with 100% methanol at 25°C for 48 h. The samples were then filtered and extracted at room temperature using a rotary evaporator.

Free radical scavenging activity assay using DPPH

The DPPH assay for antioxidant activity in crude extracts of medicinal plants was based on a previously published protocol (Shimada et al., 1992). Extracts were prepared in 0.15 μM DPPH, and 1 ml of each solution was added to a test tube. The reaction solution was mixed well and kept at room temperature for 30 min. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer.

Assay for total phenolic content

Samples (100 μl) of each extract were mixed with 1 M phenol reagent in test tubes and left to stand for 3 min after vortexing. Next, 300 μl of Na₂O₃ was added to each sample. A reagent blank was prepared using distilled water. The samples were incubated for 1 h at room temperature in the dark. The total phenolic content was then determined with a UV spectrophotometer at 725 nm using a standard curve, following the procedure for the Folin-Ciocalteu assay (Kim et al., 2007).

Assay for total flavonoid content

We used the method of Moreno et al. (2000) to measure the total flavonoid content of each sample. Samples (100 µl) were diluted to 900 µl with 80% ethanol and then mixed with 100 µl of 10% aluminum nitrate and 100 µl of 1 M potassium acetate. These samples were added to 4.3 ml of 80% ethanol and stabilized for 40 min at room temperature. All values were determined by measuring the absorbance at 415 nm using a UV spectrophotometer.

Statistics

All of the experiment were repeated at least in triplicate. The data represented the mean (SD). Significant differences between the means were assessed by using Tukey's honestly significant difference test at $P < 0.05$.

Conclusions

The effects of LED light on plants include increased plant growth and enhanced biological activity. Red LED light caused increases in plant biomass, and blue LED light enhanced the antioxidant activity of *P. multiflorum*. Therefore, it can be inferred that LED light could be important to the plant industry.

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