Effects of light intensity and LED spectrum on the medicinal component accumulation of hydroponic *Hypericum perforatum* L. under controlled environments

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Abstract: Medicinal components of *Hypericum perforatum* L. plants varies widely due to fluctuations in growth environment and biotic and abiotic contamination during cultivation management. The quality of extracts or preparations is difficult to control because of the unstable raw materials. The aim of this study is to enhance the yield and medicinal component contents of *H. perforatum* by optimizing lighting factors under controlled environment. *H. perforatum* plants were hydroponically cultivated for 30 d under 3 levels of photosynthetic photon flux density (PPFD) with 200, 300, and 400 μmol/(m^2·s) using white LEDs (R:B ratio is the ratio of red light to blue light, R:B ratio of 0.9 and 1.8) and white plus red LED (R:B ratio of 2.7). The results showed that PPFD and LED spectrum had significant effects on the growth and accumulation of medicinal components of *H. perforatum*. Biomass accumulation of stem, leaf, and root increased linearly with the increase of PPFD under each LED spectrum. Fresh weights and dry weights of stem, leaf, and root were significantly higher under a PPFD of 400 μmol/(m^2·s) with R:B ratio of 0.9 than those of 200 μmol/(m^2·s), respectively. The relative growth rate and net photosynthetic rate showed linear relationships with PPFD under the same LED spectrum. Total hypericin content, total hyperforin content, and energy yield of hypericin increased with increasing PPFD. Total hypericin content and energy yield of hypericin of P400-L0.9 were 78% and 89% more than those of P400-L2.7, respectively. Total hyperforin content and energy yield of hyperforin of P400-L0.9 and P400-L2.7 were no significant differences. Based on energy efficiency, an R:B ratio of 0.9 of white LEDs with a PPFD of 400 μmol/(m^2·s) was beneficial to improve medicinal component contents of hydroponic *H. perforatum* in plant factory with LED lighting.

Keywords: LED spectrum, light intensity, controlled environments, *Hypericum perforatum* L.
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1 Introduction

Hypericum perforatum L. is a perennial herb of the genus *Hypericum* in the Hypericaceae family. It is native to Europe, the Middle East, and North Africa, and is now widely distributed in temperate regions and higher altitudes of the tropical mountains worldwide[1]. There are up to 500 species of the genus *Hypericum* in the world[2]. The main medicinal components of *H. perforatum* are hypericin, pseudohypericin, and hyperforin, which have antidepressant, antiviral, antitumor, antibacterial, and anti-inflammatory effects. *H. perforatum* extracts are widely used for treatment of mild to moderate depression in Europe and the United States. Commercial antidepressant preparations are composed of 12% flavonoids, 4% phloroglucinols (hyperforin) and 0.3% naphthodianthrones (hypericin and pseudohypericin)[3]. In 2017, the number of patients with depression reached 264 million in the world[4]. Therefore, there is a great demand for *H. perforatum* extracts in the global market. The contents of medicinal components of *H. perforatum* plants which are currently mainly derived from wild and field cultivation are highly susceptible to the effects of photosynthetic photon flux density (PPFD)[5], light quality[6], temperature[7,8], moisture[9], and CO_2 concentration[10], resulting in the actual medicinal components contents of its commercial dietary supplements being 47%-165% of its labeled contents[11]. When harvested plant materials are used for industrial applications, the standardization of medicinal components in plants is a critical issue. Moreover, plant raw materials harvested from the field or wild are vulnerable to biotic and abiotic contamination[12]. It has previously been observed that total dry weight per plant, hypericin, pseudohypericin, and hyperforin contents (mg per plant) of *H. perforatum* grown in a controlled environment system were 2.1, 6.1, 5.6, 10.6 times greater, respectively, than those in the field in winter treatment[13]. Therefore, growing *H. perforatum* under controlled environment is an alternative method to ensure its safety and efficacy compared to field cultivation. However, lighting cost accounted for 70%-80% of the total electricity consumption in a plant factory with artificial lighting for year-round production[14]. Light-emitting diodes (LEDs) can significantly reduce electricity consumption owing to cool photon-emitting surfaces, continuous improvement of lighting efficiency, and higher light energy use efficiency[15]. Hence, it is necessary to optimize PPFD and LED spectrum for hydroponic *H. perforatum* in a plant factory.

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Light not only provides energy for photosynthesis, but also as a signal received by photoreceptors modulates a wide range of responses from germination to fruiting, a complex developmental program called photomorphogenesis[16]. Previous studies have shown that the biomass[5], development of dark glands[17], hypericins content[10,18], and hyperforin content[19] of *H. perforatum* are related to PPFD. Chou[20] revealed that the response of hyperforin content varied at different temperatures in response to PPFD. The dry weights of *H. perforatum* plants grown under red and white light at a PPFD of 500 μmol/(m^2·s) were greater than those of plants grown under blue light at a PPFD of 500 μmol/(m^2·s). Protohypericin and protopseudohypericin as precursors in *H. perforatum* plant tissue are converted into hypericin and pseudohypericin in the presence of light, respectively. The optimum wavelength for the conversion of protohypericin and protopseudohypericin in the extract of *H. perforatum* to hypericin and pseudohypericin is approximately 515 nm[21]. Sobhani Najafabadi et al.[6] demonstrated that five-week red light could promote hypericins content of adventitious root of *H. perforatum*.

However, the optimum spectrum of LEDs and PPFD for hydroponic *H. perforatum* production in plant factories with artificial lighting is not known. Therefore, this study investigated the effects of PPFD and LED spectrum on growth, photosynthetic characteristics, and major medicinal components accumulation of hydroponic *H. perforatum* to determine the appropriate light environment for efficient production in plant factories.

2 Materials and methods

2.1 Seedling materials and growth conditions

Seeds of *H. perforatum* plants (Richters Herbs, Goodwood, Canada) were placed in a culture dish, and then soaked in 100 mg/L gibberellin solution for 12 h. PPFD provided by white plus red LEDs (Beijing Lighting Valley Technology Co., Beijing, China) with R:B ratio of 1.2 was 150 μmol/(m^2·s). After treatment with gibberellin, seeds were evenly sprinkled on the filter paper of the culture dish. PPFD and LED spectrum were kept constant. Seeds were sprayed with purified water regularly every day to keep them moist. Seeds started to germinate after 6-7 d. After one month, *H. perforatum* seedlings with 4-8 true leaves were put into wet sponges (25 mm×25 mm×25 mm), and seedlings with sponges were planted in 128-hole trays (540 mm×280 mm). The nutrient solution formula used was as follows, mg/L: N-NO_3, 49.51; N-NH_4, 17.54; P, 21.70; K, 87.32; Ca, 45.12; Mg, 13.88; Fe, 2; Cu, 0.01; Mn, 0.2; Zn, 0.02; B, 0.2; Mo, 0.005. The pH of the nutrient solution was 6.0-6.5, and EC was 0.9 mS/cm. The nutrient solution was renewed once a week during the seedling stage and later cultivation stage. The light source was the same as that used for the gibberellin treatment with a PPFD of 150μmol/(m^2·s) and photoperiod of 16 h/d. Ambient air temperature and relative humidity during light/dark period were kept at (27±1)°C/(23±1)°C and (70±5)%/(65±10)%, respectively. CO_2 concentration was maintained at (400±50) μmol/mol during photoperiod and not controlled during the dark period.

2.2 LED light treatment and cultivation conditions

H. perforatum seedlings were grown under nine treatments created by combinations of three PPFDs at 200, 300, 400 μmol/(m^2·s) and three kinds of LED lamps with R:B ratios of 0.9, 1.8, 2.7 (Beijing Lighting Valley Technology Co., Beijing, China) (Table 1). The spectral distribution of the lamps was measured using a spectrometer (AvaSpec-ULS2048, Avantes Inc., The Netherlands) from 300-800 nm at 15 cm below the lamps' surface with a PPFD of 300 μmol/(m^2·s) (Figure 1). Eighteen seedlings of uniform size (total fresh weight of 101-day-old seedlings was 2.6 g per plant, about 30 cm tall) were planted in each treatment in a container-type plant factory equipped with a circulating hydroponic system (Nutrient Film Technique), and the cultivation density was 25 plants/m^2. The nutrient solution formula was consistent with that of the seedling stage. The photoperiod during the cultivation stage was 16 h/d (light time from 8:00 a.m. to 0:00 a.m.). Air temperature and relative humidity regimes were established at (25±1)°C/(20±1)°C and (65±5)%/(75±10)% (light/dark), respectively. CO_2 concentration was maintained at (800±50) μmol/mol during the photoperiod and CO_2 was not enriched during the dark period.

Table 1 Lighting treatments created by three levels of photosynthetic photon flux density (PPFD) provided by three LED lamps with R:B ratios of 0.9, 1.8, 2.7 (L) for hydroponic *H. perforatum*, respectively

Treatments	PPFD /mol·m^{-2}·s^{-1}	R:B ratio[X)]	DLI[Y)] /mol·m^{-2}·d^{-1}	Light source
P200-L0.9	200	0.9	11.5	White LED lamp with color temperature of 6500 K
P300-L0.9	300		17.3	
P400-L0.9	400		23.0	
P200-L1.8	200	1.8	11.5	White LED lamp with color temperature of 4000 K
P300-L1.8	300		17.3	
P400-L1.8	400		23.0	
P200-L2.7	200	2.7	11.5	Ratio of white chip and red chip in the LED lamp is 5:1. The color temperature of white chip is 4000 K and the peak wavelength of red chip is 660 nm
P300-L2.7	300		17.3	
P400-L2.7	400		23.0	

Note: [X)] R:B ratio is ratio of red light to blue light. [Y)]DLI is daily light integral, mol/(m^2·d), which equals photosynthetic photon flux density (μmol/(m^2·s))× photoperiod (h/d)×3600 (s/h)×10^{-6}. P200-L0.9 indicates photosynthetic photon flux density (PPFD) of 200 μmol/(m^2·s) and R:B ratio of 0.9.

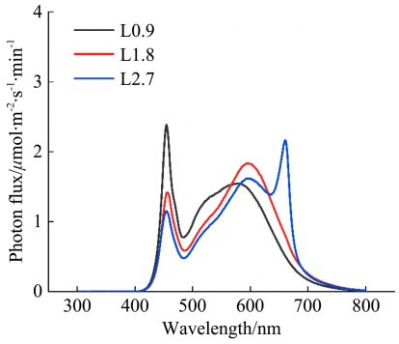


Figure 1 Spectral distribution of LED lighting environments at photosynthetic photon flux density (PPFD) of 300 μmol/(m^2·s)

Table 2 Spectral characteristics of LED lighting treatments at photosynthetic photon flux density (PPFD) of 300 μmol/(m^2·s) provided by three LED lamps with R:B ratios of 0.9, 1.8, 2.7, respectively (L0.9, L1.8, L2.7)

Spectral band/nm	Spectral fraction of light source/%		
	L0.9	L1.8	L2.7
Photon flux (300-800 nm)	100.0	100.0	100.0
Ultraviolet light (300-399 nm)	0.0	0.0	0.0
Blue light (400-499 nm)	27.1	18.7	15.8
Green light (500-599 nm)	46.6	43.8	38.7
Red light (600-699 nm)	24.1	34.3	42.5
Far-red light (700-800 nm)	2.1	3.1	2.9
R:B ratio	0.9	1.8	2.7

Note: R:B ratio is the ratio of red light to blue light.

2.3 Measurement indexes and methods

2.3.1 Determination of biomass

Six uniform *H. perforatum* plants were randomly selected for following biomass measurement 30 days after treatment in each treatment. After weighing the fresh weights of stems, leaves, and roots, dry weights of stems, leaves, and roots were determined after drying in a fan-forced oven at 105°C for 3 h and at 80°C for 72 h. The relative growth rate was calculated by referring to the method of De Groot et al.[22]: Relative growth rate=$(\ln W_2-\ln W_1)/(t_2-t_1)$. In this function, W_1 is the total dry weight of the plant at the time of planting, W_2 is the total dry weight of the plant at the time of harvest, and t_2-t_1 means the cultivation period.

2.3.2 Measurement of photosynthetic properties

Net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate of the sixth mature leaf from the top of main stem were measured using a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA) with 6400-15 extended reach 1 cm chamber. Temperature, CO_2 concentration, and flow rate of leaf chamber were maintained at 25°C, 800 mol/mol, and 500 mol/s, respectively.

2.3.3 Extractions and quantifications of hypericin and hyperforin

The extraction and chemical analysis methods of hypericin and hyperforin were modified from the method delineated by Ang et al.[23]. Leaves of the top main stem (about 10 cm long) of *H. perforatum* 30 d after treatment were cut and placed in liquid nitrogen. Then, leaves treated with liquid nitrogen were put into a vacuum freeze dryer (LGJ-10, Beijing Sihuan Scientific Instrument Co., Ltd., China) for freeze drying with a freezing temperature of −50°C, a vacuum degree of 6.2 Pa, and a freezing time of 24 h. Dried samples were weighed and ground in mortars. Powder samples (0.2 g) were extracted with 10 ml of 2% (v/v) pyridine in methanol in an ultrasonicator (SBL-10DT, Ningbo Scientz Biotechnology Co., Ltd., China) with an ice bath for 15 min. Samples were centrifuged for 15 min at 4000×*g* (TGL-16A, Hunan Pingfan Science and Technology Co., Ltd., China). The extracted solution was filtered through a 0.22 μm nylon syringe filter prior to HPLC analysis. The extraction procedure was performed under low light intensity at room temperature. A 20 μL sample of the extracted solution was injected into a Waters Symmetry C_{18} column (5 μm; 4.6 mm×250.0 mm) in an Acchrom S6000 HPLC system (Huapu Tec Inc., Beijing, China). The analytes were separated with equivalent elution using a mobile phase of 0.05 M triethylammonium acetate buffer and acetonitrile (20:80, v/v) at a flow rate of 1.0 mL/min and column temperature of 25°C. The pH of the triethylammonium acetate buffer was adjusted to 6.5. Hypericin (≥95% purity, Sigma Aldrich, USA) was quantified at 588 nm and hyperforin (≥85% purity, Sigma Aldrich, USA) at 290 nm in an Acchrom S6000 photodiode array detector (Huapu Tec Inc., Beijing, China). The standard curves were acquired by plotting the peak areas of standard concentrations of hypericin (1.0, 2.0, 4.0, 6.0, 10.0 μg/mL) and hyperforin (2.5, 5.0, 10.0, 25.0, 50.0 μg/mL). High linearity (R^2>0.99) was attained for each standard curve. The quantifications of hypericin and hyperforin were carried out based on the peak area (RT, retention times of 9.0 and 5.7 min, respectively) with comparison to the standard curves. Contents of medicinal components were expressed as mg/g DM, and the total contents of medicinal components were calculated by multiplying the contents of medicinal components by total leaf dry weight per plant.

2.3.4 Energy yield of medicinal components

Energy yield of medicinal components defined as total medicinal components contents in each treatment divided by power consumption of light source during cultivation stage is used for evaluation of LED lamps efficiency in hydroponic *H. perforatum* plant production[24].

2.4 Statistical analysis

The experiment was performed with a completely randomized design with 3×3 factorials. Statistical analysis was implemented using SPSS 20.0 software (IBM, Inc., Chicago, IL, USA). Data were analyzed by two-way analysis of variance (ANOVA) to determine the interaction effect between PPFD and light quality, and the differences between the means were tested by the least significant difference (LSD) test ($p\leq0.05$). The results were expressed as the mean±standard deviation (SD) values (n=6).

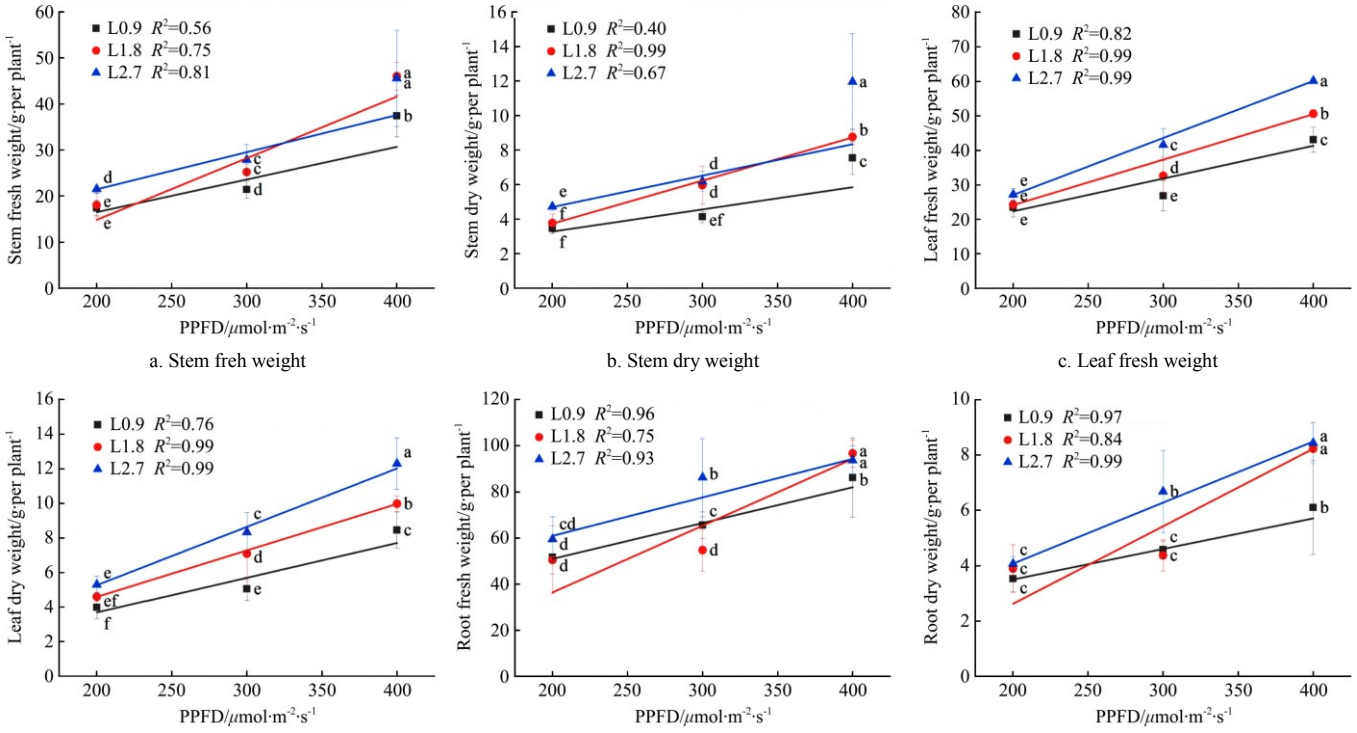
3 Results and discussion

3.1 Effects of PPFD and LED spectrum on the growth of hydroponic *H. perforatum*

PPFD and LED spectrum had significant effects on fresh weights and dry weights of stem, leaf, and root (Figure 2). The fresh weights and dry weights of stem, leaf, and root of *H. perforatum* grown under each light quality enhanced significantly when PPFD increased from 200 to 400 μmol/(m^2·s). For example, dry weights of stem, leaf, and root of *H. perforatum* grown under P400-L2.7 were 153%, 132%, and 107% greater than those of P200-L2.7, respectively (Figures 2b, 2d, and 2f). It was previously suggested that dry weights of stem, leaf, and root of *H. perforatum* were significantly higher in 500 μmol/(m^2·s) of monochromatic red and white light provided by fluorescent lamps with a sharp-cut glass filter compared to 250 μmol/(m^2·s)[5]. Similarly, Mosaleeyanon et al.[10] demonstrated that fresh weights and dry weights of stem, leaf, root, and total biomass of *H. perforatum* increased linearly as PPFD increased from 100 to 600 μmol/(m^2·s). Leaves captured more photons at high PPFD, which was responsible for the increase in biomass of *H. perforatum* in response to increasing PPFD. Stem fresh weight, root fresh weight, and root dry weight of P400-L1.8 and P400-L2.7 were significantly higher than those of P400-L0.9, but there was no significant difference between the two treatments (Figures 2a, 2e, and 2f). Stem dry weight, leaf fresh weight, and leaf dry weight of P400-L2.7 were highest among all treatments, which were 12.0 g/plant, 60.2 g/plant, and 12.3 g/plant, respectively (Figures 2b, 2c, and 2d). Stem dry weight, leaf fresh weight, and leaf dry weight of P400-L2.7 were 0.6, 0.4, and 0.5 times greater than those of P400-L0.9, respectively. Previous evidence of the promotion of *H. perforatum* growth associated with monochromatic red light and white light was provided by Nishimura[5], where plants grown under monochromatic red light and white light at 500 μmol/(m^2·s) had more dry weights of leaf, stem, and root than those under monochromatic blue light. In the same way, leaf dry weight of sweet basil increased with R:B ratio ranging from 0.5 to 4.0[25]. This was mainly due to more blue photons could reduce leaf area by reducing cell expansion[26].

Environmental variables also had a great influence on the relative growth rate controlled by genes. The relative growth rate was closely related to PPFD. *H. perforatum* exhibited significantly greater relative growth rate in response to increasing PPFD from 200 to 400 μmol/(m^2·s) (Figure 3). The average relative growth rate of 400 μmol/(m^2·s) was 23% higher than that of 200 μmol/(m^2·s). It was previously suggested that the relative growth rate of spinach under 190 μmol/(m^2·s) was 1.1 times greater than that of spinach under 90 μmol/(m^2·s), but the relative growth

rate would decrease with continuous increase in PPFD[27]. The relative growth rate of R:B ratio of 2.7 with a PPFD of 400 μmol/(m^2·s) was significantly higher than those of R:B ratio of 0.9 and 1.8 with a PPFD of 400 μmol/(m^2·s), which was 142.96 mg/(g·d). The results showed that more red photons were favorable to promote the relative growth rate of *H. perforatum*.



a. Stem freh weight b. Stem dry weight c. Leaf fresh weight

d. Leaf dry weight e. Root fresh weight f. Root dry weight

Figure 2 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on biomass accumulation of *H. perforatum*

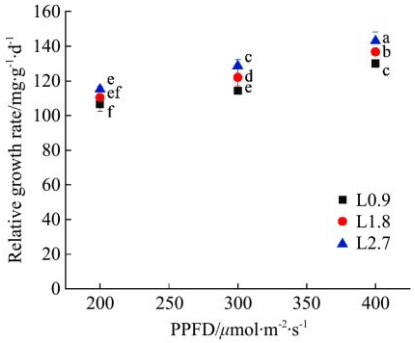


Figure 3 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on relative growth rate of *H. perforatum*

3.2 Effects of PPFD and LED spectrum on photosynthetic characteristics of hydroponic *H. perforatum*

Net photosynthetic rate, stomatal conductance, and transpiration rate of *H. perforatum* were affected significantly by PPFD and LED spectrum besides intercellular CO_2 concentration (Table 3). The net photosynthetic rate of *H. perforatum* increased with increasing PPFD under each light quality. For instance, net photosynthetic rate of R:B ratio of 0.9 increased by 108% when PPFD increased from 200 to 400 μmol/(m^2·s). Consistent with our finding, Mosaleeyanon et al[10] revealed that the net photosynthetic rate of *H. perforatum* at different CO_2 concentrations (500, 1000, and 1500 μmol/mol) increased significantly when PPFD increased from 100 to 600 μmol/(m^2·s). The net photosynthetic rate of P400-L2.7 (16% blue light) was 53% higher than that of P400-L0.9 (27% blue light). This finding confirmed that net photosynthetic rate of corn leaf tended to decrease with increasing blue light percentage from 0% blue light to 100% blue light[28]. The stomatal conductance of *H. perforatum* under R:B ratio of 0.9 and 2.7 increased dramatically with the increase of PPFD up to 300 μmol/(m^2·s), but it had not continued to increase at a PPFD of 400 μmol/(m^2·s). Hattori et al.[29] also observed a significant increase in stomatal conductance of rice with increasing PPFD. The intercellular CO_2 concentration of R:B ratio of 1.8 decreased with the increase of PPFD up to 400 μmol/(m^2·s). With the exception of R:B ratio of 1.8, transpiration rate of *H. perforatum* followed the same trend with stomatal conductance.

Table 3 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on photosynthetic characteristics of *H. perforatum* leaves

Treatments	Net photosynthetic rate /μmol·m^{-2}·s^{-1}	Stomatal conductance /mmol·m^{-2}·s^{-1}	Intercellular CO_2 concentration /μmol·mol^{-1}	Transpiration rate /mmol·m^{-2}·s^{-1}
P200-L0.9	2.5±0.4^{f}	283±117^{c}	778±8^{ab}	1.00±0.36^{d}
P300-L0.9	3.8±0.7^{e}	533±175^{b}	780±6^{ab}	1.50±0.33^{c}
P400-L0.9	5.2±0.3^{c}	600±292^{b}	775±11^{b}	1.66±0.58^{bc}
P200-L1.8	3.1±0.4^{f}	575±150^{b}	785±3^{a}	1.88±0.22^{bc}
P300-L1.8	4.5±0.4^{d}	550±176^{b}	778±3^{ab}	1.82±0.43^{bc}
P400-L1.8	8.0±0.7^{a}	533±52^{b}	765±4^{c}	1.83±0.10^{bc}
P200-L2.7	3.5±0.3^{e}	633±121^{b}	783±3^{a}	1.98±0.20^{b}
P300-L2.7	6.4±0.4^{b}	921±361^{a}	780±7^{ab}	2.82±0.65^{a}
P400-L2.7	7.9±0.2^{a}	881±55^{a}	778±1^{ab}	3.08±0.04^{a}
PPFD	*	*	*	*
LQ	*	*	NS	*
PPFD×LQ	*	*	*	*

3.3 Effects of PPFD and LED spectrum on medicinal components accumulation of hydroponic *H. perforatum*

Hypericin is synthesized in the dark glands of *H. perforatum*[17]. Hyperforin is synthesized in translucent glands of *H. perforatum*[30]. PPFD and LED spectrum had a significant

effect on hypericin content (Figure 4). Hypericin content increased linearly with the increase of PPFD under each light quality. Hypericin content of P400-L0.9 was 10 times greater than that of P200-L0.9, which was 0.77 mg/g. Our study supported evidence from previous results verified by Briskin et al.[18] that every 70 to 100 μmol/(m^2·s) increase in PPFD yielded around a 1.2 to 1.5-fold increase in *H. perforatum* leaf hypericin content. Similarly, an increase in PPFD from 802 to 1619 μmol/(m^2·s) resulted in a linear increase in hypericin content of *H. perforatum*[19]. Photosynthetic products in the plant can be roughly divided into three destinations: 1) for regrowth; 2) converted into reusable storage materials such as starch and protein; 3) for synthesis of secondary metabolites. On the basis of overflow metabolism concept, when the carbohydrates produced by plants surpass the carbon requirement linked with plant growth, the additional carbohydrates are transported into the biosynthesis of secondary metabolites[31]. In the current study, the increase in carbon assimilation arose from the increase in photons harvested by *H. perforatum* leaves under 400 μmol/(m^2·s) could result in more secondary metabolites. In addition to R:B ratio of 2.7, hyperforin content of R:B ratio of 0.9 and 1.8 decreased with increasing PPFD (Figure 4). Hyperforin content of P200-L1.8 was 84% higher than that of P400-L1.8. In accordance with the present results, previous studies have demonstrated that hyperforin content at a PPFD of 250 μmol/(m^2·s) was 1.6-1.9 times higher than that of 500 μmol/(m^2·s)[5]. In our study, hypericin content of P400-L0.9 was 196% more than that of P400-L2.7. This supported previous findings that one-week blue light treatment could stimulate synthesis of hypericin of adventitious root of *H. perforatum*[6]. There were no significant differences in hypericin contents among three LED spectra treatments with a PPFD of 200 μmol/(m^2·s). This also accorded with previous observations, which showed that hypericin contents of *H. perforatum* leaves under monochromatic blue light, monochromatic red light, and white light with a PPFD of 250 μmol/(m^2·s) had no significant differences[5]. Hyperforin content of P200-L0.9 was 1.1 times greater than that of P200-L2.7. This result further supported the idea that hyperforin content of *H. perforatum* leaves under blue light with a PPFD of 150 μmol/(m^2·s) was greater than that of white light[32]. These results showed that more blue photons could be conducive to the synthesis of medicinal components of *H. perforatum*.

Total hypericin content was also significantly affected by PPFD and LED spectrum (Figure 5). Total hypericin content increased linearly with the increase of PPFD under each light quality. The total hypericin content of P400-L0.9 was higher than other treatments, which was 6.12 mg/plant. The total hypericin content of P400-L2.7 was 44% less than that of P400-L0.9. Total hyperforin content of R:B ratio of 0.9 and 2.7 increased with increasing PPFD except for R:B ratio of 1.8. Total hyperforin contents of P400-L0.9 and P400-L2.7 were greater than other treatments, which were 1188 and 1164 mg/plant. These discrepancies were mainly attributed to the increase of leaf dry weight with increasing PPFD under each LED spectrum and P400-L2.7 treatment had more leaf dry weight. These results agree with the findings of Mosaleeyanon et al.[10], in which total hypericin content and total hyperforin content of *H. perforatum* increased significantly with increasing PPFD ranging from 100 to 600 μmol/(m^2·s).

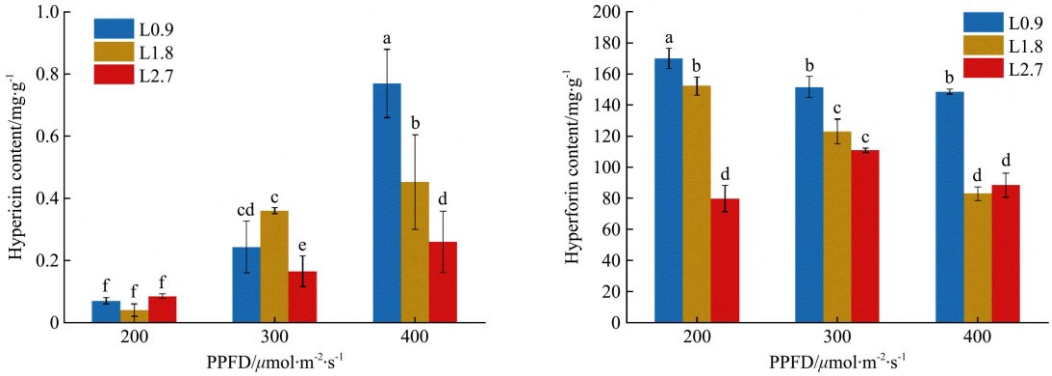


Figure 4 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on medicinal component contents of *H. perforatum* leaves

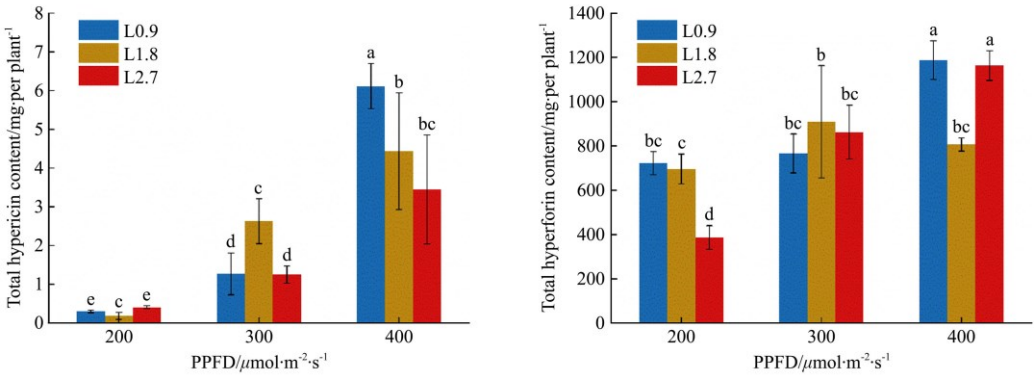


Figure 5 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on total medicinal component contents of *H. perforatum* leaves

3.4 Effects of PPFD and LED spectrum on energy yield of medicinal components of hydroponic *H. perforatum*

Energy yield of hypericin was significantly affected by PPFD and LED spectrum (Figure 6). Energy yield of hypericin increased with increasing PPFD under each light quality. Compared to P200-L0.9, energy yield of hypericin of P400-L0.9

increased by 11 times. Energy yield of hypericin of P400-L0.9 (1.57 mg/kWh) was higher than other treatments and was 89% more than that of P400-L2.7. This indicated that P400-L0.9 could produce more hypericin when consuming the same electric energy compared to other treatments. Energy yield of hyperforin was not significantly influenced by PPFD and LED spectrum.

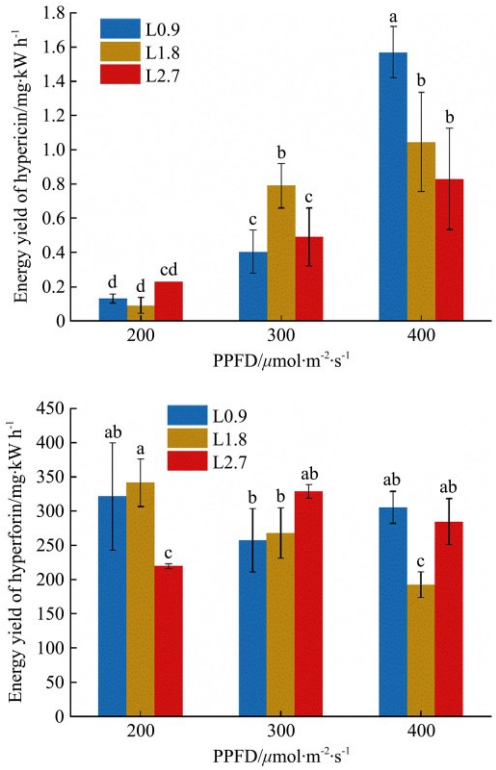


Figure 6 Effects of photosynthetic photon flux density (PPFD) and LED spectrum on energy yield of medicinal components of *H. perforatum* leaves

4 Conclusions

The optimizing light environment can promote the accumulation of biomass and medicinal components of *H. perforatum*. The increase of PPFD could stimulate photosynthetic capacity and biomass accumulation of *H. perforatum*. Under the same LED spectrum, total hypericin content, total hyperforin content and energy yield of hypericin increased with the increase of PPFD. R:B ratio of 0.9 with a PPFD of 400 μmol/(m^2·s) can be used for hydroponic *H. perforatum* production in plant factories.

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[References]

[1] Crockett S L, Robson N K B. Taxonomy and chemotaxonomy of the genus hypericum. Medicinal and Aromatic Plant Science and Biotechnology, 2011; 5(S1): 1–13.

[2] Savran H, Koyuncu O, Yaylacı Ö K. All aspects of *Hypericum perforatum* (St. John's wort). International Journal of Environmental Research and Technology, 2019; 2(3): 126–134.

[3] De Carvalho Meirelles G, Bridi H, Rates S M K, von Poser G L. Southern brazilian *Hypericum* species, promising sources of bioactive metabolites. Elsevier, 2018; 59: 491–507.

[4] James S L, Abate D, Abate K H, Abay S M, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the global burden of disease study 2017. The Lancet, 2018; 392(10159): 1789–1858.

[5] Nishimura T, Zobayed S M A, Kozai T, Goto E. Medicinally important secondary metabolites and growth of *Hypericum perforatum* L. Plants as affected by light quality and intensity. Environmental Control in Biology, 2007; 45(2): 113–120.

[6] Sobhani Najafabadi A, Khanahmadi M, Ebrahimi M, Moradi K, Behroozi P, Noormohammadi N. Effect of different quality of light on growth and production of secondary metabolites in adventitious root cultivation of *Hypericum perforatum*. Plant Signaling & Behavior, 2019; 14(9): 1640561.

[7] Zobayed S M A, Afreen F, Kozai T. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. Plant Physiology and Biochemistry, 2005; 43(10-11): 977–984.

[8] Kaundal M, Sharma R, Kumar R. Elevated CO_2 and temperature effect on growth, phenology, biomass and hypericin content of *Hypericum perforatum* L. Plant Physiology Reports, 2021; 26: 116–127.

[9] Zobayed S M A, Afreen F, Kozai T. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. Environmental and Experimental Botany, 2007; 59(2): 109–116.

[10] Mosaleeyanon K, Zobayed S M A, Afreen F, Kozai T. Relationships between net photosynthetic rate and secondary metabolite contents in St. John's wort. Plant Science, 2005; 169(3): 523–531.

[11] Constantine G, Karchesy J. Note variations in hypericin concentrations in *Hypericum perforatum* L. and commercial products. Pharmaceutical Biology, 1998; 36(5): 365–367.

[12] Zobayed S M A, Afreen F, Kozai T. Necessity and production of medicinal plants under controlled environments. Environmental Control in Biology, 2005; 43(4): 243–252.

[13] Mosaleeyanon K, Zobayed S M A, Afreen F, Kozai T. Enhancement of biomass and secondary metabolite production of St. John's wort (*Hypericum perforatum* L.) under a controlled environment. Environmental Control in Biology, 2006; 44(1): 21–30.

[14] Kozai T, Niu G. Overview and concept of closed plant production system (CPPS). In: Kozai T, Niu G and Takagaki M (Eds.), editors. Plant factory: An indoor vertical farming system for efficient quality food production. Elsevier Academic Press, 2016; pp.3–5.

[15] He D X, Kozai T, Niu G, Zhang X. Light-emitting diodes for horticulture. In: Li J and Zhang G (Eds.), editors. Light-Emitting Diodes Materials, Processes, Devices and Applications, Cham, Switzerland: Springer, 2019; pp.513–547.

[16] Carvalho R F, Takaki M, Azevedo R A. Plant pigments: the many faces of light perception. Acta Physiologiae Plantarum, 2011; 33(2): 241–248.

[17] Zobayed S M A, Afreen F, Goto E, Kozai T. Plant-environment interactions: accumulation of hypericin in dark glands of *Hypericum perforatum*. Annals of Botany, 2006; 98(4): 793–804.

[18] Briskin D P, Gawienowski M C. Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. Plant Physiology and Biochemistry, 2001; 39(12): 1075–1081.

[19] Odabas M S, Raduğieneuml J, Camas N, Janulis V, Ivanauskas L. The quantitative effects of temperature and light intensity on hyperforin and hypericins accumulation in *Hypericum perforatum* L. Journal of Medicinal Plants Research, 2009; 3(7): 519–525.

[20] Chou Y C. Study on the contents of active ingredients in St. John's wort (*Hypericum perforatum* L.) under different cultivation conditions and their extraction with microwave-assisted method. Master dissertation. Taichung: National Chung Hsing University, 2014. 85p.

[21] Poutaraud A, Di Gregorio F, Tin V C F, Girardin P. Effect of light on hypericins contents in fresh flowering top parts and in an extract of St. John's wort (*Hypericum perforatum*). Planta Medica, 2001; 67(3): 254–259.

[22] De Groot C C, Marcelis L F, Van Den Boogaard R, Lambers H. Growth and dry-mass partitioning in tomato as affected by phosphorus nutrition and light. Plant, Cell & Environment, 2001; 24(12): 1309–1317.

[23] Ang C Y, Cui Y, Chang H C, Luo W, Heinze T M, Lin L J, et al. Determination of St. John's wort components in dietary supplements and functional foods by liquid chromatography. Journal of AOAC international, 2002; 85(6): 1360–1369.

[24] Chung H Y, Chang M Y, Wu C C, Fang W. Quantitative evaluation of

electric light recipes for red leaf lettuce cultivation in plant factories. HortTechnology, 2018; 28(6): 755–763.

[25] Hikosaka S, Moriyama F, Goto E. Effects of photosynthetic photon flux density and red/blue light ratio on the leaf shape and concentrations of functional and aromatic compounds in sweet basil (*Ocimum basilicum* L.). The Horticulture Journal, 2021: UTD–273.

[26] Dougher T A, Bugbee B. Long-term blue light effects on the histology of lettuce and soybean leaves and stems. Journal of the American Society for Horticultural Science, 2004; 129(4): 467–472.

[27] Nguyen T, Tran T, Nguyen Q. Effects of light intensity on the growth, photosynthesis and leaf microstructure of hydroponic cultivated spinach (*Spinacia oleracea* L.) under a combination of red and blue leds in house. International Journal of Agricultural Technology, 2019; 15(1): 75–90.

[28] Zhen S, Bugbee B. Steady-state stomatal responses of C_3 and C_4 species to blue light fraction: Interactions with CO_2 concentration. Plant, Cell & Environment, 2020; 43(12): 3020–3032.

[29] Hattori T, Sonobe K, Inanaga S, An P, Tsuji W, Araki H, et al. Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. Environmental and Experimental Botany, 2007; 60(2): 177–182.

[30] Soelberg J, Jørgensen L B, Jäger A K. Hyperforin accumulates in the translucent glands of *Hypericum perforatum*. Annals of Botany, 2007; 99(6): 1097–1100.

[31] Matsuki M. Regulation of plant phenolic synthesis: From biochemistry to ecology and evolution. Australian Journal of Botany, 1996; 44(6): 613–634.

[32] Karimi M, Ahmadi N, Ebrahimi M. Red LED light promotes biomass, flowering and secondary metabolites accumulation in hydroponically grown *Hypericum perforatum* L. (cv. Topas). Industrial Crops and Products, 2022; 175: 114239. doi: 10.1016/j.indcrop.2021.114239.