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Growth and Bioactive Compounds of *Salvia plebeia* R. Br. Grown under Various Ratios of Red and Blue Light

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Abstract: We investigated the effects of red and blue light on the growth and content of bioactive compounds of *Salvia plebeia* R. Br in a closed-type plant production system (CPPS). The seedlings of *Salvia plebeia* R. Br. were transplanted into a deep floating technique system with nutrient recycling (pH 6.5 and electrical conductivity (EC) 1.5 dS·m^{-1}). The plants were cultured for a duration of 35 days at 25 ± 1 °C, with relative humidity 60 ± 5%, a 12/12 h (light/dark) photoperiod, and a light intensity of 180 µmol·m^{-2}·s^{-1} photosynthetic flux photon density, providing standard fluorescent (FL) lighting and various light qualities of red:blue ratios (10:0, 7:3, 5:5, 3:7, and 0:10) in the CPPS. The growth characteristics of *Salvia plebeia* R. Br., such as leaf length, leaf area, and fresh and dry weights of shoots, were the greatest in Red only and R7B3. The leaf shape index was the highest in Blue only and specific leaf weight was lower in FL and Blue than in the other treatments. The photosynthetic rate was the highest in R7B3. The total phenolic and flavonoid concentrations per gram of fresh weight of *Salvia plebeia* R. Br. were higher in combined light, such as R7B3, R5B5, and B3B7, than in the monochromatic light treatments. However, the antioxidant activity per fresh weight was the highest in FL. In conclusion, the results suggest that 7:3 is the most effective red and blue light ratio for production of high quality *Salvia plebeia* R. Br. in a CPPS.

Keywords: combined light; specific leaf area; total phenolic content

1. Introduction

Salvia plebeia R. Br. is an annual or biennial plant belonging to the Lamiaceae family. The leaves are oval or lanceolate, their stems are square-shaped, and it is native to warm and humid areas in Korea, Japan, and China. The main bioactive compounds of *Salvia plebeia* R. Br. include flavonoids, polyphenols, saponin, cardiac glycosides, and unsaturated sterols [1,2], as well as luteolin-7-O-glucoside, caffeic acid, hispidulin, rosmarinic acid, nepetin, hispidulin-7-O-glucoside [3], plebeianiol A, and diterpenoids [4]. Typically, young leaves of *Salvia plebeia* R. Br. are eaten as a vegetable and used as a crude drug. The extracts of the plant are reported to have respiratory protection effects [5], fat cell differentiation and fat accumulation inhibitory effects [6], anti-inflammatory effects [7], anti-diabetes mellitus effects [8], anti-cancer effects [2], and antioxidant effects [9]. In addition, it is known to be effective as a treatment for immune diseases. Although it can be used as a material for functional foods, studies of mass production cultivation techniques are lacking.

Light is an important element that controls plant growth, development, and metabolism through light quality, light intensity, light direction, and photoperiod, and is an essential energy source for photosynthesis [10,11]. Light quality, which designates color or wavelength, strongly influences the physiological, morphological, and biochemical parameters of plants more than light intensity or photoperiod [12–14]. The different wavelength ranges, such as visible light (400–700 nm), which is called photosynthetic active radiation (PAR), ultraviolet rays (UV), and far-red, can be used for various purposes as well as plant photosynthesis [15]. Previous studies indicated that the apparent photomorphogenic responses of plants to light quality are controlled by networks of multiple photoreceptors, including phytochromes, phototropins, and cryptochromes [16,17]. Phytochromes perceive red and far-red light, whereas blue light is absorbed by cryptochromes and phototropins [18,19]. Therefore, red light (600–700 nm) and blue light (400–500 nm) were reported to serve as essential sources for photosynthesis [14], since these wavelengths coincide with the photosynthetic pigments absorption peak [20]. Red and blue light also have an important impact on plant growth and development [21,22].

Plant responses to red light generally include seed germination, cell development and elongation, fresh and dry weight increases, and increase in leaf area [23–25]. The plant response to blue light includes stomatal opening, compact growth, and flavonoid accumulation [26,27]. However, monochromatic red or blue light is insufficient for the requirements of normal plant growth. The net photosynthetic rate, chlorophyll content, and fresh weight of plants grown under monochromatic light were lower compared to under red light supplemented with blue light [28–31]. Monochromatic red light decreased leaf area and delayed opening of flowers on cucumber seedlings after transplanting [32]. Johkan et al. [23] reported that lettuce had a smaller leaf area and lower fresh weight when grown under monochromatic light compared with combined red and blue light.

Hence, the objective of this study was to investigate the influence of red and blue light irradiation on the growth, photosynthetic rate, and bioactive compound accumulation for the cultivation of the medicinal plant, *Salvia plebeia* R. Br., in a closed-type plant production system.

2. Materials and Methods

2.1. Plant Materials and Light Treatment

Seeds of *Salvia plebeia* R. Br. (Asia seed Co. Ltd., Seoul, Korea) were sown in 128-hole plug trays filled in formed medium (Terra-plug, Smithers-Oasis Co. Ltd., Cheonan, Korea) in a closed-type plant production system (CPPS; C1200H3, FC Poibe Co. Ltd., Seoul, Korea) at a temperature of 25 ± 1 °C, with a relative humidity of 60 ± 5%, photoperiod of 12/12 h (light/dark), and light intensity of 180 μmol·m^{-2}·s^{-1} photosynthetic photon flux density. The 40-day-old seedlings were transplanted into a deep floating technique system with recycling multipurpose nutrient solution ($(CaNO_3)_2 \cdot 4H_2O$ 472.0, KNO_3 202.0, KH_2PO_4 272.0, NH_4NO_3 80.0, $MgSO_4 \cdot 7H_2O$ 246.0, Fe-EDTA 15.0, H_3BO_3 1.4, $MnSO_4 \cdot 4H_2O$ 2.1, $ZnSO_4 \cdot 7H_2O$ 0.8, $CuSO_4 \cdot 5H_2O$ 0.2, $Na_2MoO_4 \cdot 2H_2O$ 0.1 mg·L^{-1}, pH 6.5, and electrical conductivity (EC) 1.0 dS·m^{-1}) in a CPPS. The plants were cultured for a duration of 35 days under the same growth conditions as the germination conditions. The light treatments, sourced from fluorescent lamps (FL, FHF32SSEX-D, Osram Co. Ltd., Munich, Germany) and red and blue light emitting diodes (LEDs; ES LEDs Co. Ltd., Seoul, Korea), were as follows: FL (control), Red (red:blue = 10:0), Blue (red:blue = 0:10), R7B3 (red:blue = 7:3), R5B5 (red:blue = 5:5), and R3B7 (red:blue = 3:7). The light spectral distributions of the experiment were measured using a spectroradiometer (ILT950, International Light Technologies Inc., Peabody, MA, USA) at 5 points (center and four edges, Figure 1), and the light intensity (mean with five places) was measured using a photometer (HD2101.2, Delta Ohm SrL, Caselle, Italy).

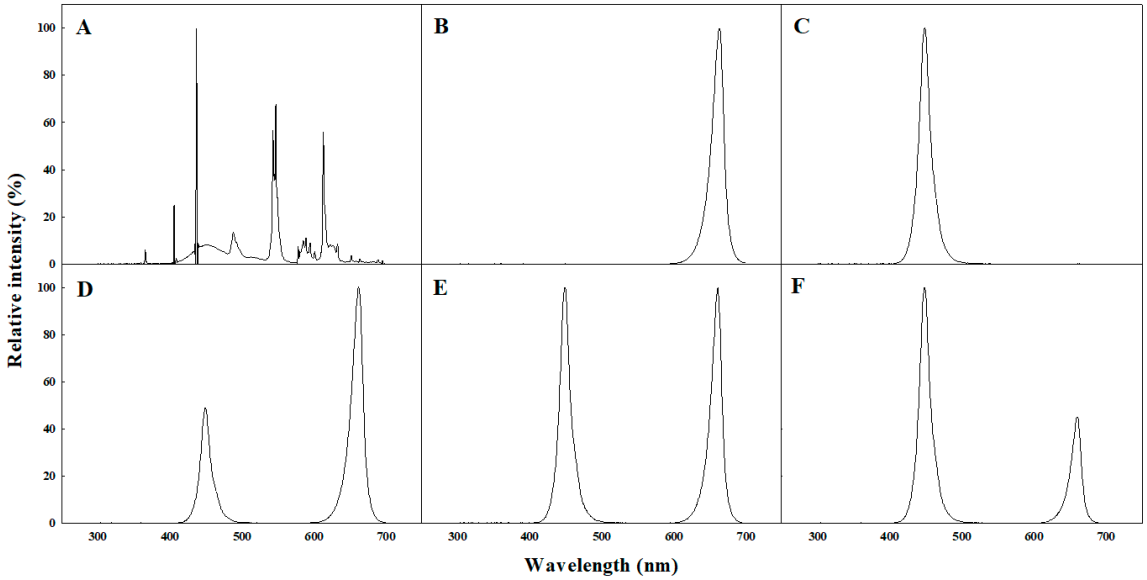

Figure 1. Relative spectral distribution of light used in a closed-type plant production system. (**A**) fluorescent lamp; (**B**) Red (red:blue = 10:0); (**C**) Blue (red:blue = 0:10); (**D**) R7B3 (red:blue = 7:3); (**E**) R5B5 (red:blue = 5:5); and (**F**) R3B7 (red:blue = 3:7).

2.2. Growth Characteristics

At 35 days after initiating light treatments, the leaf length, leaf width, fresh weight (FW) and dry weight (DW) of shoots and roots, and leaf area were measured. Leaf area was measured using a leaf area meter (LI-3000, LI-COR Inc., Lincoln, NE, USA). The FW was measured using an electronic balance (EW220-3NM, Kern & Sohn GmbH., Balingen, Germany) and the DW was measured after drying in an oven (Venticell-220, MMM Medcenter Einrichtunger GmbH., Planegg, Germany) at 70 °C for 72 h. The leaf epinasty index was used following Fukuda [33], and calculated as:

$$\text{Leaf epinasty index } = 1 - 0.5\left(\frac{\text{LD}}{\text{LW}}\right) \quad (1)$$

where LD is the distance between the two edges of the leaf at the level of the maximum width and LW is the maximum leaf width when flat. The specific leaf weight (SLW) and leaf shape index were calculated using:

$$\text{SLW (mg·cm}^{-2}\text{)} = \text{dry weight of leaf (mg)/total leaf area (cm}^2\text{)} \quad (2)$$

$$\text{Leaf shape index} = \text{leaf length/leaf width} \quad (3)$$

The chlorophyll content was measured using a portable chlorophyll meter (SPAD-502, Konica Minolta Inc., Tokyo, Japan). Photosynthetic rate was measured using a portable photosynthesis system (CIRAS-3, PP Systems International Inc., Amesbury, MA, USA) on a completely unfolded fifth leaf from the apex. The measurement conditions were controlled as follows: air flow rate 150 mL·min^{-1}, leaf area 4.5 mm^2, leaf temperature 25 °C, CO_2 concentration 500 µmol·mol^{-1}, and 180 µmol·m^{-2}·s^{-1} photosynthetic photon flux density (PPFD).

2.3. Bioactive Compounds

2.3.1. Total Phenolics

A 1 g leaf sample was immediately frozen in liquid nitrogen and then finely ground. To extract phenolic compounds, the sample was mixed with 5 mL of 80% methanol. The mixture was shaken for 24 h at room temperature and then centrifuged at 10,509× *g* for 10 min. After centrifugation, the supernatant was used to determine total phenolic concentration [34]. The total phenolic concentration of *Salvia plebeia* R. Br. was determined using the Folin–Ciocalteu reagent method

of Singleton and Rossi [34]. Approximately 200 µL of extract was mixed with 300 µL distilled water and 250 µL 2N Folin–Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO, USA). The mixture was then combined with 1.25 mL of 20% Na_2CO_3, vortexed for 5 s, and incubated at room temperature for 20 min. The absorbance of the supernatant was measured with a spectrophotometer (Libra S22, Biochrom Ltd., Cambridge, UK) at 735 nm to determine the total phenolic concentration, which is expressed as µg gallic acid equivalent (GAE) per mg of FW.

2.3.2. Total Flavonoids

The extraction method for the total flavonoid concentration of the sample was the same as for total phenolic concentration. The total flavonoid concentration of the leaf was determined using a method modified from Kumaran and Karunakaran [35]. A volume of 900 µL of 80% methanol and 1 mL of 2% $AlCl_3$ were added to 100 µL of the extract. After vortexing for 5 s, the mixture was reacted at room temperature for 40 min. The absorbance of the samples was measured with a spectrophotometer at 415 nm to determine the total flavonoid concentration, which is expressed as µg quercetin equivalent (QE) per mg of FW.

2.3.3. Antioxidant Activity

The extraction method for antioxidant activity was the same as for total phenolic concentration. The antioxidant activity of *Salvia plebeia* R. Br. was determined according to a modified 2,2-diphenyl-1picrylhydrazyl (DPPH, Sigma-Aldrich Co., St. Louis, MO, USA) method for free radical scavenging activity [36]. To 100 µL of the extract, 1 mL of 0.3 mM DPPH was added. After vortexing for 5 s, the mixture was reacted at room temperature for 20 min in dark conditions. The absorbance of the samples was measured using a spectrophotometer at 517 nm and the scavenging activity of DPPH free radicals was calculated as:

$$\text{Scavenging activity } (\%) = \left(1 - \frac{\text{absorbace of sample at 517 mm}}{\text{absorbace of control at 517 mm}}\right) \times 100 \quad (4)$$

To determine the antioxidant activity of samples, a standard curve was prepared using 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and DPPH free radicals scavenging activity (%) calculated using the above equation was converted to activity in terms of µg Trolox equivalents (TE) per mg FW of the sample.

2.4. Statistical Analysis

The experiments were repeated 3 times with 10 plants per repetition for each treatment, each in a randomized complete block design. After selecting plants of uniform size, 9 plants per treatment were used to determine plant growth parameters. The bioactive compounds were measured using 6 plants per treatment. Statistical analysis was carried out using the Statistical Analysis System program (SAS 9.1, SAS Institute Inc., Cary, NC, USA). Experiment results were subjected to analysis of variance (ANOVA) and Tukey's multiple range tests. The SigmaPlot program was used for graphing (SigmaPlot 12.0, Systat Software Inc., San Jose, CA, USA).

3. Results and Discussion

3.1. Growth Characterics

The leaf characteristics of *Salvia plebeia* R. Br. as affected by various light treatment at 35 days after transplanting are shown in Figure 2A. The leaf growth characteristics, such as leaf length, leaf width, leaf area, and SLW, tended to be higher in the treatments with a high ratio of red to blue light, such as Red and R7B3; in the treatments with a high ratio of blue light, Blue and R3B7, these values were lower. The leaf length and leaf width were the greatest in Red and R7B3, at 12.3 and 6.4 cm, respectively (Figure 2B,C). Similarly, the leaf area was the largest in R7B3 at 843.7 cm^2, and the smallest

in FL (control) at 382.0 cm^2 (Figure 2D). The SLW, representing leaf thickness, was the lowest in FL and Blue (Figure 2E). Red light is known to develop the chlorophyll by stimulating phytochrome, which is a photoreceptor that plays an essential role in the growth and development of plants [37,38]. Although the effect of light quality on plant growth and morphology may vary depending on plant species, many studies have shown that red light elongates the plant and blue light inhibits plant height [39,40]. In this study, red light promoted leaf growth. In addition, *Salvia splendens* F. exhibited increased leaf area, plant height, and shoot dry weight in monochromatic red light compared to monochromatic blue light [41].

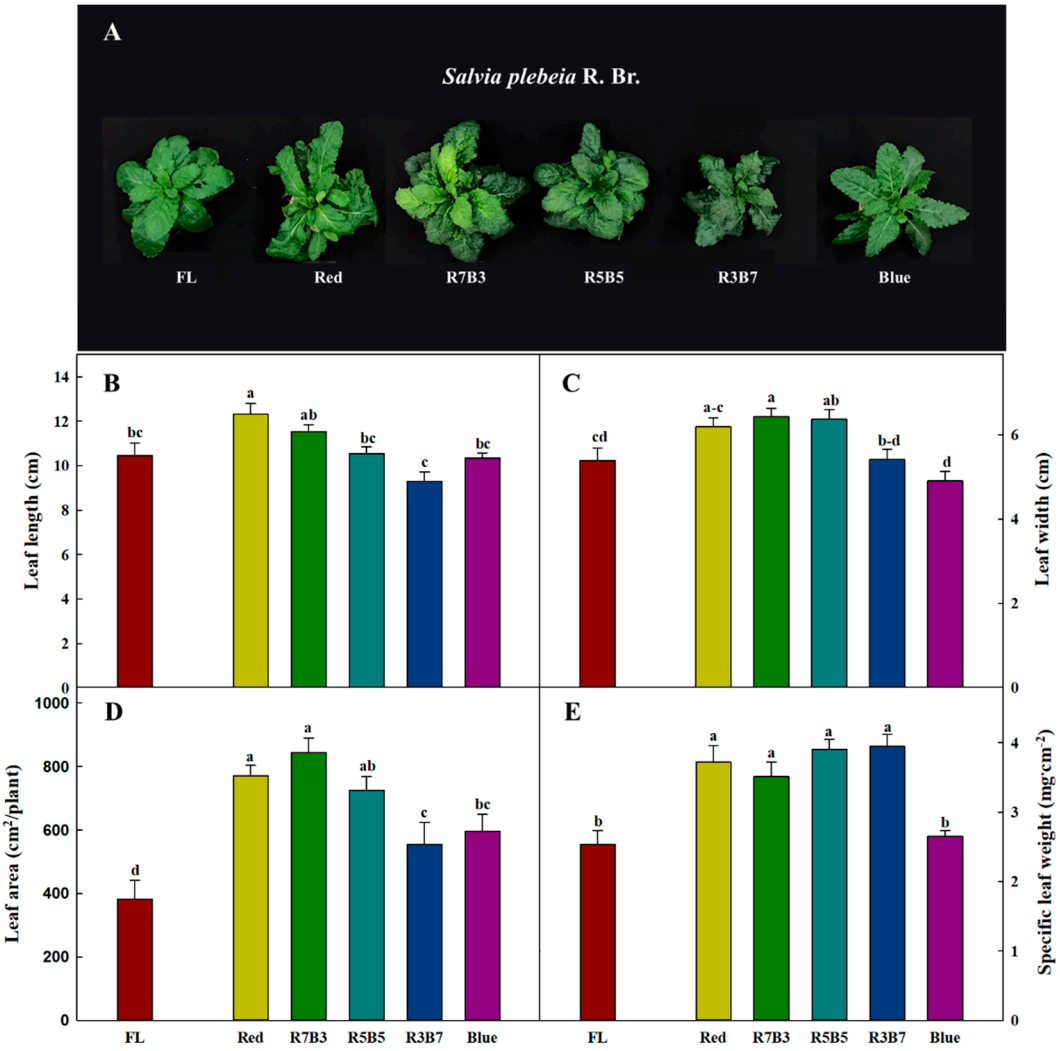

Figure 2. The growth (**A**), leaf length (**B**), leaf width (**C**), leaf area (**D**), and specific leaf weight (**E**) of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting. Vertical bars represent the standard deviation from the mean (n = 3). Different letters in the same column indicate significant differences based on Tukey's multiple range test ($\alpha \leq 0.05$).

The leaf epinasty index of *Salvia plebeia* R. Br. was the lowest in Blue (Figure 3A). The leaf epinasty index indicates the degree of leaf epinasty as follows: 0, closed; 0.5, open horizontally; and 1.0, close to the abaxial side. Epinasty, in which the edges of the leaves droop down, does not occur under blue light alone, so red light is considered to be closely related to epinasty. These results are consistent with the results of a study on red light inducing epinasty in *Pelargonium zonale* Ait. (geranium) [33]. In addition, Lee et al. [42] reported the epinasty of *Spinacia oleracea* (spinach) in red and mixed light. The leaf shape index of *Salvia plebeia* R. Br. as affected by light quality was lowest in R5B5 and R3B7 (Figure 3B). The higher leaf shape index was due to a longer and narrower leaf, and the shorter and

broader leaf shapes indicated the lower leaf shape index. Previous studies reported that red light leads to long leaves in *Lactuca sativa* L. (lettuce) [43,44], which is similar to the results in this study.

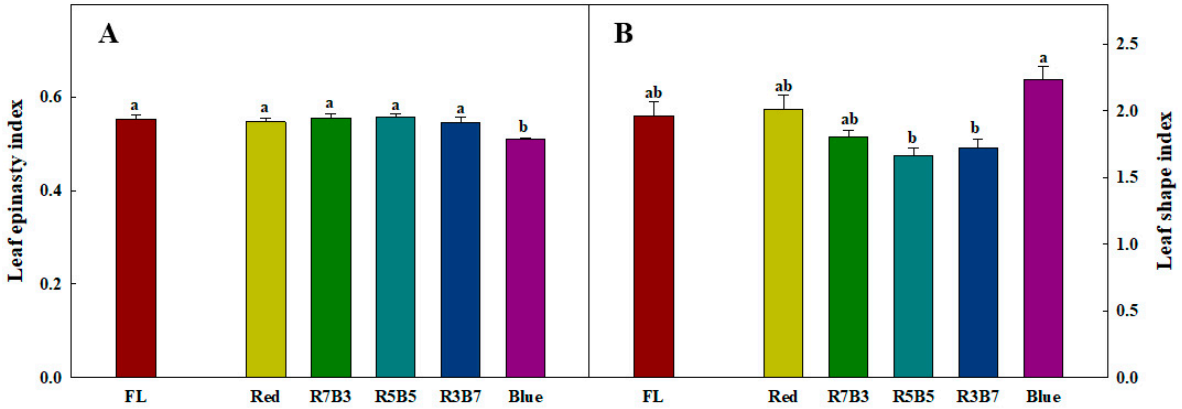

Figure 3. Leaf epinasty index (**A**) and leaf shape index (**B**) of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting. Vertical bars represent the standard deviation from the mean (n = 3). Different letters in the same column indicate significant differences based on Tukey's multiple range test ($\alpha \leq 0.05$).

The fresh and dry weights of shoots and roots of *Salvia plebeia* R. Br. are shown in Table 1. The fresh weight of shoots was the greatest in Red and R7B3 at 31.3 and 33.5 g, respectively. Fresh and dry weights of shoots were the lowest in FL at 11.3 and 0.9 g, respectively. In Red, the fresh and dry weights of roots increased by 3.7 and 4.0 times that of FL, respectively. Red light can be used effectively in photosynthesis during leaf development and lead to the accumulation of starch [45], increasing the biomass of plants [23,46]. Several studies have reported that red light increased the fresh and dry weights of shoots in various crops, such as *Solanum lycopersicum* L. (tomato) seedlings, *Perilla frutescens* L., lettuce, and *Mesembryanthemum crystallinum* L. (ice plant) [25,47–49].

Table 1. Fresh and dry weights of shoots and roots of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting (n = 3).

Light Quality [z]	Fresh Weight (g/plant)		Dry Weight (g/plant)	
	Shoot	Root	Shoot	Root
FL	11.3 c [y]	5.8 d	0.9 d	0.3 c
Red	31.3 a	21.3 a	2.8 ab	1.2 a
R7B3	33.5 a	11.4 b	3.0 a	0.9 ab
R5B5	25.2 ab	10.4 bc	2.9 ab	0.9 ab
R3B7	20.0 b	6.5 cd	2.2 bc	0.5 bc
Blue	17.4 bc	10.4 bc	1.6 cd	0.6 bc
Significance	***	***	***	***

[z] Refer to Figure 1 for details on the light quality. [y] Mean followed by different letters are significantly different by Tukey's multiple range at $\alpha \leq 0.05$. *** ANOVA F significant at $p \leq 0.001$.

3.2. SPAD Value and Photosynthetic Parameters

The soil-plant analysis development (SPAD) value, which indicates chlorophyll content, is shown in Figure 4. The SPAD value was the highest in R5B5 at 54.5 and the lowest in Blue at 41.2. Red and blue light influence energy provision, the accumulation of pigments, such as chlorophyll and carotenoid, secondary pigments involved in photosynthetic CO_2 fixation, and basic metabolism [50,51]. The chlorophyll content of *Brassica oleracea* var. *italica* L. (kale) and *Brassica oleracea* var. *acephala* DC (broccoli) grown under monochromatic blue light was reported to be reduced [52,53]. In contrast, other studies have reported that blue light increased the chlorophyll content of lettuce and *Brassica pekinensis* Rupr. (Chinese cabbage) [23,31]. The chlorophyll content affected by light quality

was indicated depending on varieties. To investigate the photosynthetic ability of *Salvia plebeia* R. Br. under the different light treatments, the photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration were measured at 35 days after transplanting.

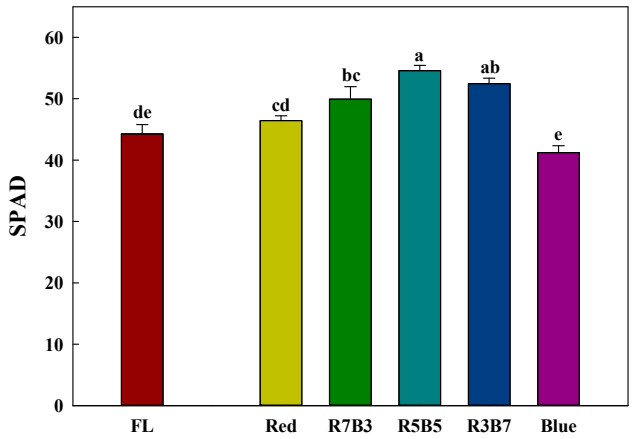

Figure 4. SPAD (soil-plant analysis development) values of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting. Vertical bars represent the standard deviation from the mean (n = 3). Different letters in the same column indicate significant differences based on Tukey's multiple range test ($\alpha \leq 0.05$).

The photosynthetic rate was significantly higher in R7B3 at 5.7 µmol CO_2 $m^{-2} \cdot s^{-1}$. Blue had the lowest photosynthetic rate, only 54% of that of R7B3 (Figure 5A). The stomatal conductance and transpiration rate were lower in FL compared with LED treatments (Figure 5B,C). The intercellular CO_2 concentration was the opposite of the photosynthetic rate: Blue was 1.3 times more than R7B3 (Figure 5D).

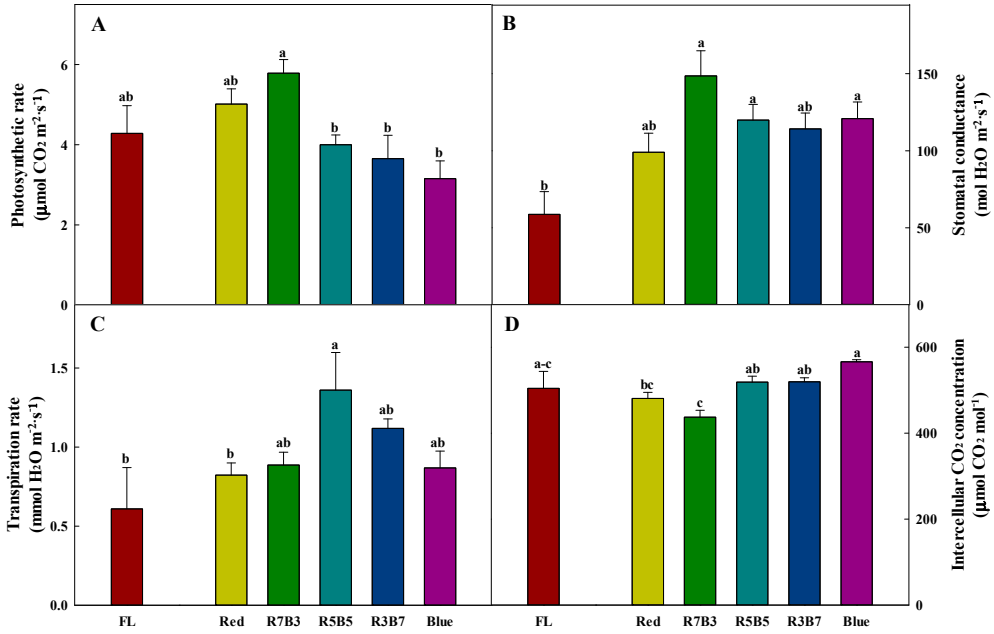

Figure 5. Photosynthetic rate (**A**), stomatal conductance (**B**), transpiration rate (**C**), and intercellular CO_2 concentration (**D**) of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting. Vertical bars represent the standard deviation of the mean (n = 3). Different letters in the same column indicate significant differences based on Tukey's multiple range test ($\alpha \leq 0.05$).

The combination of red and blue light and far-red irradiation more efficiently promote photosynthesis than monochromatic light [54], producing a synergistic effect via phytochromes

and crytochromes [55]. Some studies reported that the combination of red and blue light effectively increased the dry weight of lettuce compared with monochromatic red light [27,56]. Stomatal conductance can be expressed as an indicator of the degree of stoma opening and closure in leaves. CO_2, which is essential for photosynthesis, moving through the stomata suggests that stomatal conductance affects photosynthetic rate. In this study, when the stomatal conductance was high, the photosynthetic rate was also high. Also, Roni et al. [57] reported that with a high photosynthetic rate, demand for CO_2 was high, leading to a lower intercellular CO_2 concentration in *Eustoma* leaves, and that blue light had a higher intercellular CO_2 concentration compared with red light under same light intensity. This is because at a higher assimilation rate, more intercellular CO_2 is converted to photosynthetic products.

3.3. Bioactive Compounds

To investigate possible differences in the bioactive compound content of *Salvia plebeia* R. Br. under various light qualities, the concentrations of total phenolics, total flavonoids, and antioxidant activity were investigated at 35 days after transplanting. The bioactive compounds per unit FW had the lowest concentration in monochromatic red light (Figure 6A,C,E). The total phenolic concentration per gram FW was 1.3 times higher in R3B7 than Red.

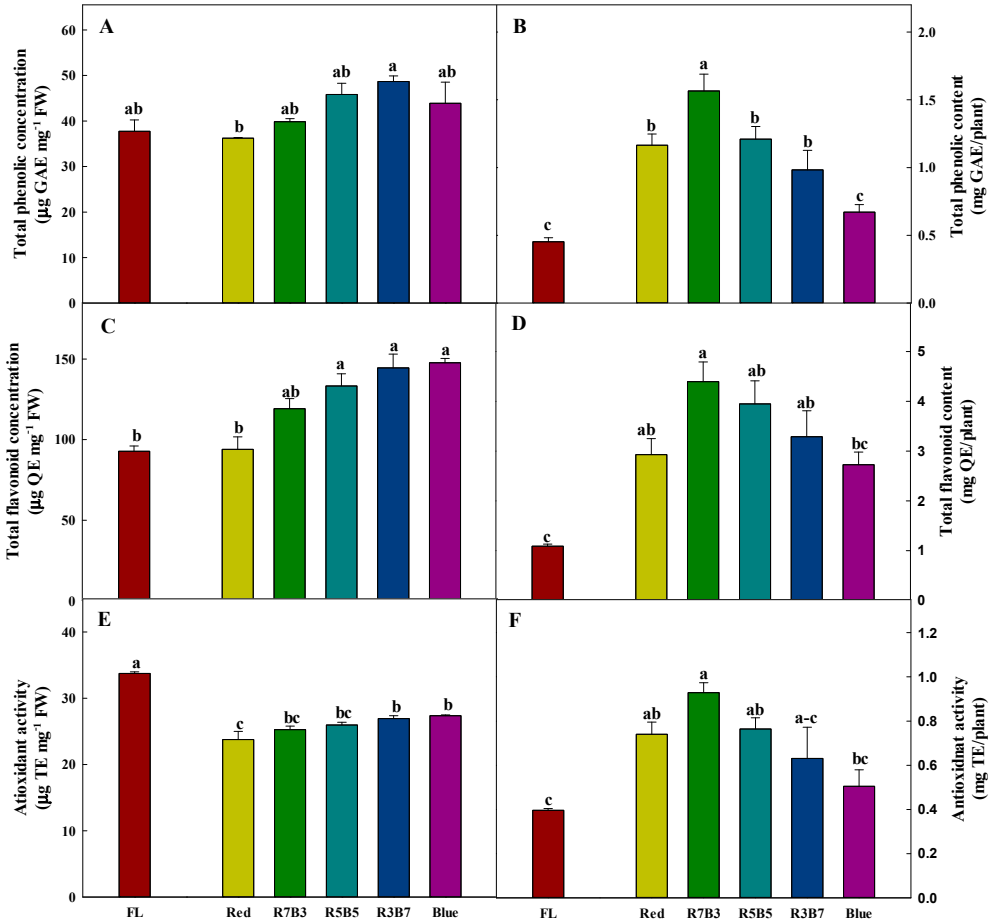

Figure 6. Total phenolic and total flavonoid concentration, and antioxidant activity, per fresh weight (**A**), (**C**), and (**E**), respectively) and total phenolic and total flavonoid content, and antioxidant activity, per plant (**B**), (**D**), and (**F**), respectively) of *Salvia plebeia* R. Br. as affected by different light treatments at 35 days after transplanting. Vertical bars represent the standard deviation from the mean (n = 3). Different letters in the same column indicate significant differences based on Tukey's multiple range test ($\alpha \leq 0.05$).

The total flavonoid concentration per gram FW was the lowest in FL and Red at 92.7 and 93.9 $\mu g \cdot mg^{-1}$, respectively. The antioxidant activity per gram FW was the highest in FL. R7B3, which had a high fresh weight and tended to have the highest content of bioactive compounds per plant (shoot) (Figure 6B,D,F). In R7B3, the contents of total phenolics, total flavonoids, and antioxidant activity per plant were 3.3, 4.0, and 2.3 times higher than in FL, respectively. Phytochemicals are known to have many benefits for human health, such as anti-allergenic, anti-inflammatory, and anti-viral activities [58–60]. Their accumulation and biosynthesis are affected by many growth conditions, such as light, temperature, nutrient solution, and CO_2 [61–63]. In particular, light is associated with the biosynthesis of secondary metabolites as well as plant growth. It is known that red light promotes the development of photosynthesis-related organs of leaves and the accumulation of starch [45,64], and blue light affects the accumulation of chemical components on plants, chloroplast development, and the formation of chlorophyll [65]. In previous studies, blue light induced the accumulation of total phenolic, anthocyanin, and chlorogenic acid in lettuce, and myo-inositol and pinitol in ice plants [23,44,66]. Park et al. [67] reported that the total phenolic and hydroxycinnamic acid concentration per DW showed no significant difference between combined light and monochromatic light in *Crepidiastrum denticulatum* (Houtt.) Pak & Kawano, but content per shoot was higher in combined light, which showed better growth than monochromatic light. Also, Spalholz et al. [68] reported that the total phenolic content of lettuce was 1.3 times higher in a combination of red and blue light in monochromatic red or blue light. Hernández et al. [69] grew tomato seedlings under various red and blue light ratios and found that seedlings in red and blue combination light treatments had 2–3 times greater anthocyanin concentration than in monochromatic blue light. In this study, similar to the results of previous studies, the content of bioactive compounds per plant was higher in the combination light, with better growth than with monochromatic light. The antioxidant activity per FW tended to be high in FL, suggesting that the FL spectrum can stimulate antioxidant activity. Park et al. [70] also reported that DPPH radical scavenging activity of *Salvia plebeia* R. Br. was higher in FL than white, red, and blue light.

4. Conclusions

Various light qualities affected the growth, morphogenesis, photosynthesis, and accumulation of bioactive compounds of *Salvia plebeia* R. Br. In Red and R7B3, which were subject to high ratios of red light, the biomass increased. In addition, red light induced the elongation of leaves, which had the highest leaf length and leaf area. Blue light induced thin and long leaves, with no leaf epinasty. The photosynthetic rate and bioactive compound content per plant were the highest in R7B3. These results indicate that R7B3, a 7:3 ratio of red to blue light, is suitable for cultivating high quality *Salvia plebeia* R. Br. in a CPPS.

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