JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Influences of Four Different Light-Emitting Diode Lights on Flowering and Polyphenol Variations in the Leaves of Chrysanthemum (*Chrysanthemum morifolium*)

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ABSTRACT: Light-emitting diodes (LEDs) are an efficient alternative to traditional lamps for plant growth. To investigate the influence of LEDs on flowering and polyphenol biosynthesis in the leaves of chrysanthemum, the plants were grown under supplemental blue, green, red, and white LEDs. Flower budding was formed even after a longer photoperiod than a critical day length of 13.5 h per day under blue light illumination. The weights of leaves and stems were highest under the white light illumination growth condition, whereas the weight of roots appeared to be independent of light quality. Among nine polyphenols characterized by high-performance liquid chromatography−tandem mass spectroscopy, three polyphenols were identified for the first time in chrysanthemum. A quantitation and principal component analysis biplot demonstrated that luteolin-7-*O*-glucoside (**2**), luteolin-7-*O*-glucuronide (**3**), and quercetagetin-trimethyl ether (**8**) were the highest polyphenols yielded under green light, and dicaffeoylquinic acid isomer (**4**), dicaffeoylquinic acid isomer (**5**), naringenin (**7**), and apigenin-7-*O*-glucuronide (**6**) were greatest under red light. Chlorogenic acid (**1**) and 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone (**9**) were produced in similar concentrations under both light types. The white and blue light appeared inefficient for polyphenol production. Taken together, our results suggest that the chrysanthemum flowering and polyphenol production are influenced by light quality composition.

KEYWORDS: *Chrysanthemum morifolium, light emitting diode, high-performance liquid chromatography−tandem mass spectrometry, principal component analysis biplot, polyphenols*

INTRODUCTION

Light is the most important environmental factor for plant growth and development. Tailoring light wavelength, photon flux (quantity), and photoperiod allows for adjusting stem elongation, flowering time, biomass accumulation, and nutritional quality. Among them, light wavelength is particularly critical for plant growth. Thus, blue light regulates phototropism, chloroplast migration, stomatal opening, leaf expansion, and photoprotection.[1,2] Green light induces leaf growth and stem elongation, decreases biomass production,[3] and participates in the photosynthetic process.[4] Red light is associated with development of the photosynthetic apparatus and the transport of assimilates.[5] In addition, red light illumination results in biomass accumulation in marigold seedlings[6] and effectively induces the callus in Cymbidium orchids.[7]

Light sources such as fluorescent, metal halide, high-pressure sodium, and incandescent lamps are generally used for plant growth under greenhouse conditions.[8] However, these sources show several disadvantages such as low photosynthetic photon flux as well as limited lifetime of operation, low quantum yield, and less suitable wavelength spectra for plant growth.[9] In 1990, light-emitting diodes (LEDs) were investigated for the first time for plant growth[10] and demonstrated to be an efficient alternative to traditional lamps used in lighting systems.[11] Compared with conventional lamps, the improved features of LEDs include smaller size and weight, solid-state construction, long lifetime (about 100 000 h), low emitting temperature, wavelength specificity, and consumption of much less energy than other sources.[12,13] Additionally, specific wavelengths within a narrow spectral range can be set with LEDs to precisely tune spectral quality and light intensity.[13,14] The physiological and morphological effects of LEDs have been studied widely for a number of plants, including potato,[15] lilium,[16] Cymbidium,[17] lettuce,[18,19] Eucalyptus,[20] spinach,[21] strawberry,[22] and *Arabidopsis*,[23] as well as marigold and Salvia bedding plants.[6]

Chrysanthemum morifolium (Chrysanthemum) are commercially important flowering herbs and short-day plants. They belong to the genus *Chrysanthemum* constituting approximately 30 species of perennial flowering plants in the family

Received: May 23, 2012
Revised: September 10, 2012
Accepted: September 12, 2012
Published: September 12, 2012

Asteraceae, which is native to Asia and northeastern Europe. Chrysanthemum was first cultivated in China as far back as the 15th century B.C.[24] Chrysanthemums are grown year-round in greenhouses in northern latitudes. Since it is one of the flowering crops sensitive to the photoperiod as well as the light quality, comprehensive understanding about the influence of the photoperiod as well as the light quality may be important for the improvement of its production efficiency. Only two studies have been carried out to show the influence of LED quality on the morphogenesis of chrysanthemum.[25,26]

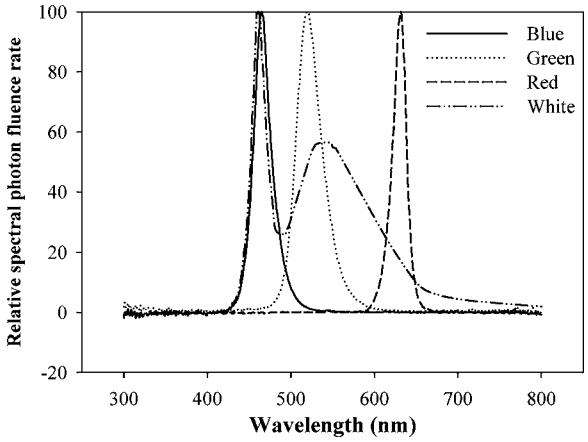

Figure 1. Spectral distributions in relative energy of the LED arrays.

Table 1. Characteristics of Flower Buds Induction and Flowering Development for Chrysanthemum Grown for 35 Days under Different Supplemental Light Qualities[a]

	blue	green	red	white
flowering (%)	100	0	0	0
days to visible flower buds	20.3 ± 0.7	NB[a]	NB	NB

[a]NB; no visible flower buds. [a]Data represent the mean ± SD (n = 30).

Six studies[27−32] on the polyphenol characterization of chrysanthemum flowers and two studies on those of its leaf[33,34] have been reported up to now. Plant secondary metabolomes such as polyphenols are known widely to function as physiological defense materials against a broad spectrum of environmental stressors including light.[35,36] To the best of our knowledge, there have been no reports on the physiological role of polyphenols in short-day plants such as chrysanthemum. Accordingly, it will be the first step for the research of physiological response of the chrysanthemum to the spectrum of different lights to disclose the role of the plant polyphenols.

The major objective of this study was to evaluate the influence of four different colored LED lights on chrysanthemum flowering and to comprehensively determine the polyphenol level variations in leaves using high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS). In addition, a principal component analysis (PCA) biplot was constructed to comprehensively describe the relationship between the illumination of different LEDs and polyphenol concentration.

MATERIALS AND METHODS

Materials and Chemicals. The block rooted cuttings of *Chrysanthemum morifolium* Ram. cv. 'Gaya yellow' were obtained from Gyeongnam Agricultural Research and Extension Services (GARES), Republic of Korea in September 2011. The cuttings were authenticated as having a homozygous genetic background by Dr. Chae-Shin Lim of GARES. The cuttings (7 ± 1 leaves per plant) were planted in plastic pots (10 cm diameter) containing a commercial media 'Tosilee' (Shinan Grow Co., Jinju, Republic of Korea) and were acclimated for 5 days under white fluorescent light (16 h, 70 ± 5 μmol/m^2/s). After acclimation, the plants were grown independently in different light treatment chambers at 20 ± 0.2 °C and 65 ± 2% humidity until the harvest date (35 days after light

Figure 2. Comparative flower buds induction of chrysanthemum grown under different supplemental light qualities. (Photos were taken 24 d after light treatments.)

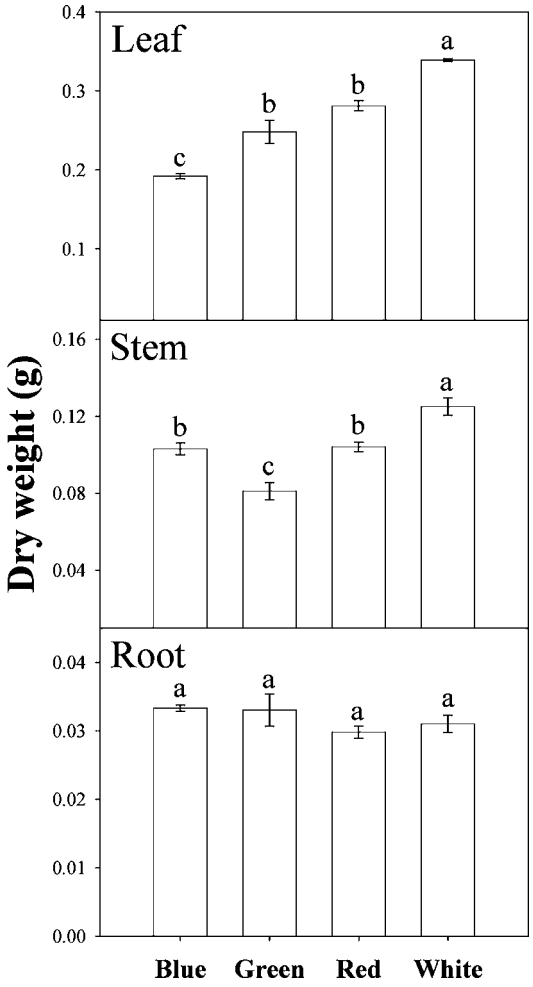

Figure 3. Dry matter partitioning of chrysanthemum grown for 35 days under different supplemental light qualities. Error bar represent SD (n = 15), and different letters indicate a significant difference (Tukey's test, $P < 0.05$).

treatment). The white fluorescent light (70 ± 5 μmol/m^2/s) was maintained for 12 h, and then each of the blue, green, red, and white lights was irradiated at 70 ± 5 μmol/m^2/s for 4 h using LED arrays (DR LED Networks Co., Seoul, Republic of Korea). The spectral energy distribution of four different LED arrays was measured from 300 to 800 nm with a spectroradiometer (International Light, RPS-900, U.S.). Their maximum spectral wavelengths were 463 (blue), 518 (green), and 632 nm (red); the white LEDs had a broad spectrum (Figure 1). Irradiance was measured using a quantum sensor (LI-COR, LI-191, Lincoln, NE, U.S.). Water was supplied daily with top irrigation and a nutrient solution (Hoagland, pH = 5.9 ± 0.2, electrical conductivity = 1.2 dS/m) every 4 days until harvest.

Flower bud differentiation was confirmed daily, and bud differentiation was investigated using microscopy. After harvest, leaves samples were stored at −70 °C, and plants were dried in an oven at 70 °C for 72 h.

HPLC grade acetonitrile and methanol and pure water were purchased from Duksan Pure Chemical Co. Ltd. (Ansan, Republic of Korea). Caffeic acid, quercetin dihydrate, naringin, luteolin, apigenin, and emodin, used as calibration standards, were purchased from Sigma-Aldrich Co. (St. Louis, MO, U.S.). The standards were recrystallized in methanol, and the purity was confirmed to be >99% by HPLC.

Extraction. The sampled chrysanthemum leaves were lyophilized (PVTED50A, Ilsin Bio Base Co. Ltd., Yangju, Republic of Korea). An 80% aqueous methanol (20 mL) solution was poured into the lyophilized samples (0.2 g), homogenized using a Polytron blender (Brinkman Instruments, Westbury, NY, U.S.) for 2 min, and extracted with a sonicator (100 W, 42 kHz, Bransonics 3510RDTH, Danbury, CT, U.S.) for 10 min at room temperature. The extracts were combined and centrifuged for 10 min at 4000 × g. The supernatant was filtered with a cellulose membrane (0.45 μm), transferred to a vial, and stored at −20 °C until analysis.

HPLC−MS/MS. HPLC−MS/MS experiments were performed according to a method described previously,[37] except for the solvent system and detection wavelength. The solvent consisted of 0.1% aqueous acetic acid (A) and acetonitrile (B). The gradient conditions for mobile phase were from 10% to 40% B for 60 min and decreased to 10% B over 10 min, followed by isocratic elution. The detection wavelength was 254 nm.

Statistical Analysis. All determinations were performed in triplicate. All results are expressed as mean ± standard deviation. Significant differences between treatment means ($P < 0.05$) were determined via a one-way analysis of variance using SAS version 9.1.3 (SAS Institute, Cary, NC, U.S.). PCA-biplot was performed with SIMCA-P 12.0.1 software (Umetrics, Umeä, Sweden).

RESULTS AND DISCUSSION

Flowering and Plant Growth. After acclimation of the chrysanthemum cuttings, each photoperiod was set to 16 h per day, in which a white fluorescent light was illuminated for 12 h, followed by treatment with the individual blue, green, red, and white lights for 4 h. While flower buds started to develop under blue light illumination after 20.3 days, no buds were induced under green, red, or white light (Table 1 and Figure 2). Chrysanthemum started to flower under a photoperiod shorter than a critical day length of 13.5 h per day, as a longer photoperiod prevents flowering.[38] However, our result suggests that flowering does not follow the day length response threshold under blue light supplementation. It has been reported that exposing chrysanthemum to blue light-supplemented long days at low light intensity does not adversely affect flower initiation and that the flowering is initiated regardless of photoperiod.[39] No flower buds were induced under green, red, or white light illumination even until 35 days. This result was similar with previous findings[40] that red or white light delays flowering in *Chenopodium rubrum* L. The reason why chrysanthemum flowering was started under a photoperiod shorter than a critical day length is not clear at present. Whether the result of blue light treatment is due to (a) the "induction" of flower bud formation or (b) "no effect or the ineffective repression or the delay" of flower bud formation should be investigated in another study.

The weights of the dried chrysanthemum tissues (leaves, stems, and roots) cultivated under the different supplemental light conditions were measured in 15 plants to compare growth properties. The dry weights were highest for the leaves grown under the supplemental white light and lowest for those grown under blue light (Figure 3). The lowest leaf weight grown under the blue light may be associated with flower production, as there is a negative relationship between flower production of photoperiod-sensitive plants and their leaf growth.[41] Dry weight was highest for stems grown under white light supplementation, followed by those grown under red, blue, and green light. The green light supplementation appeared not to greatly affect stem growth. Green light, which has a relatively higher reflectance compared to that of red or blue light, is not photosynthetically effective.[42] Root weight appeared to be independent of light quality.

Separation and Characterization of Polyphenols. A mixture of polyphenols was isolated from chrysanthemum leaves by extraction with 80% aqueous methanol. The isolated components were identified by HPLC using a C_{18} column, MS/MS, and a comparison with literature data. The HPLC chromatograms of the chrysanthemum leaves grown under the various light conditions are

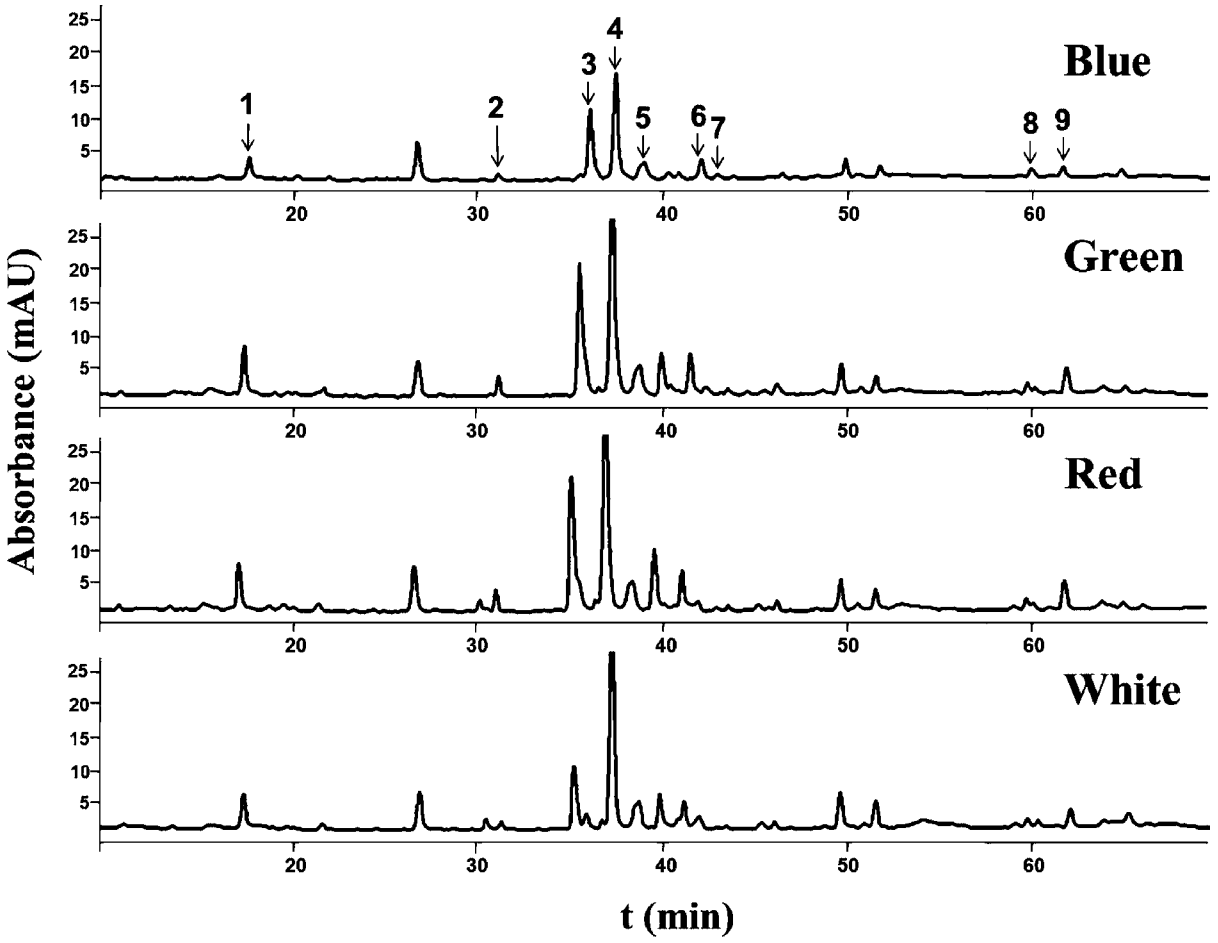

Figure 4. HPLC chromatograms of the polyphenols isolated from chrysanthemum leaves grown under different supplemental light qualities. (**1**) chlorogenic acid; (**2**) luteolin-7-*O*-glucoside; (**3**) luteolin-7-*O*-glucuronide; (**4**) dicaffeoylquinic acid isomer; (**5**) dicaffeoylquinic acid isomer; (**6**) apigenin-7-*O*-glucuronide; (**7**) naringenin; (**8**) quercetagetin-trimethyl ether; (**9**) 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone. Detection wavelength: 254 nm.

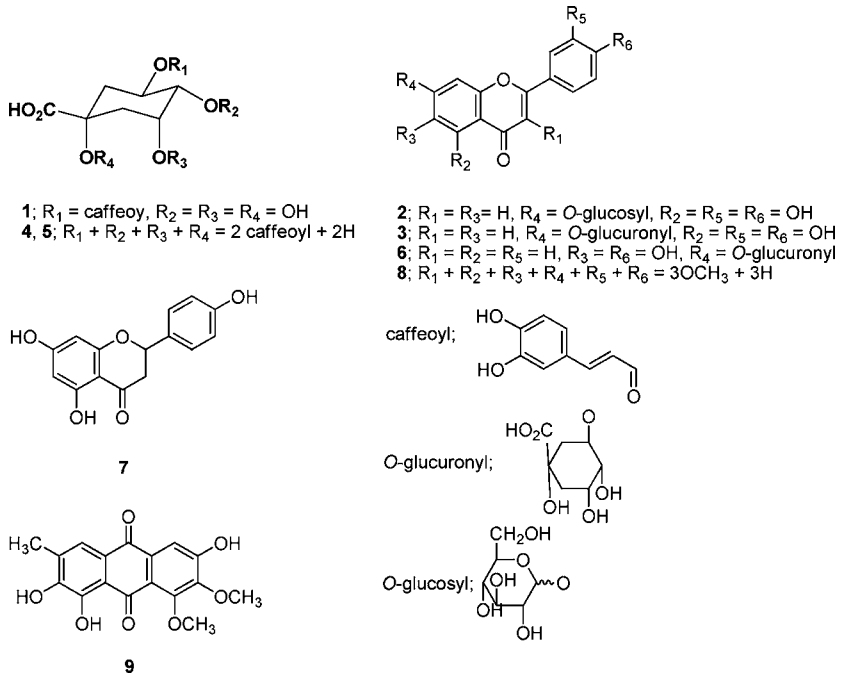

Figure 5. Structures of nine polyphenols characterized for chrysanthemum leaves grown under different supplemental light qualities.

dx.doi.org/10.1021/jf302272x | *J. Agric. Food Chem.* 2012, 60, 9793−9800

Table 2. Mass Spectral Data of Nine Polyphenols Characterized for Chrysanthemum Leaves Grown under Different Supplemental Light Qualities

r.t.[a]	$[M-H]^-$/ $[M+H]^+$[b]	MS/MS	compds	ref
17.2	353/	191, 179	chlorogenic acid (**1**)	32
32.1	/449	287, 153	luteolin-7-*O*-glucoside (**2**)	32
35.3	/463	287, 259, 153	luteolin-7-*O*-glucuronide (**3**)	32
37.4	515/	353, 191, 179, 173	dicaffeoylquinic acid isomer (**4**)	32
38.9	515/	353, 191, 179, 173	dicaffeoylquinic acid isomer (**5**)	32
43.1	/447	271, 243, 153	apigenin-7-*O*-glucuronide (**6**)	27, 53
43.8	271/	177, 165, 151, 119, 107, 93	naringenin (**7**)	43
60.1	359/	344, 329	quercetagetin-trimethyl ether (**8**)	44
62.1	329/	314, 299, 271, 199	1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone (**9**)	45

[a]r.t: retention time (min). [b]Data were obtained at negative or positive ion mode.

shown in Figure 4. Nine polyphenols were labeled in the 10–70 min absorbance segment of the chromatograms recorded at 254 nm. The structures and HPLC–MS/MS data of the nine polyphenols are shown in Figure 5 and Table 2. Six polyphenols (**1**–**6**) were reported previously in chrysanthemum,[27,32] and three polyphenols (**7**–**9**) were characterized for the first time in chrysanthemum.

Polyphenol **7** (t_R = 43.8 min) yielded $[M-H]^-$ at *m*/*z* 271, which was fragmented to yield a *m*/*z* 164 ($[M-H]^-$–$C_6H_4O_2$, retro-Diels–Alder fragment), 119 (C_8H_7O), 107 ($[M-H]^-$–$C_9H_8O_3$, retro-Diels–Alder fragment), and 93 (C_6H_5O). This component was identified as naringenin.[43] Polyphenol **8** (t_R = 60.1 min) was identified as a quercetagetin-trimethyl ether based on $[M-H]^-$ at *m*/*z* 359, which was fragmented to produce a fragment ion at 344 ($[M-H]^-$–CH_3), 329 ($[M-H]^-$–$2CH_3$), and 314 ($[M-H]^-$–$2CH_3$).[44] Polyphenol **9** (t_R = 62.1 min) was 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone. Its MS/MS spectrum consisted of $[M-H]^-$ at *m*/*z* 329, which fragmented to 314 ($[M-H]^-$–CH_3), 299 ($[M-H]^-$–$2CH_3$), 271 ($[M-H]^-$–$2CH_3$–CO), and 243 ($[M-H]^-$–$2CH_3$–2CO).[45]

Quantification. The nine chrysanthemum polyphenols were quantified from peak chromatogram areas extracted at 254 nm. The quantification was validated in terms of specificity, linearity, recovery, precision, limit of detection (LOD), and limit of quantification (LOQ), according to the guidelines of the International Conference of Harmonization.[46] The validation data are shown in Table 3. Specificity is noninterference with other analytes detected in the region of interest. As shown in Figure 4, the analyte peaks were well separated without any other peaks interfering, indicating good specificity. Polyphenols **1**–**9** were quantified using the linear calibration curves of structurally related compounds. Thus, caffeic acid derivatives **1**, **4**, and **5** were quantified as caffeic acid, luteolin derivatives **2** and **3** as luteolin, apigenin-7-*O*-glucuronide (**6**) as apigenin, naringenin (**7**) as naringin, quercetagetin-trimethyl ether (**8**) as quercetin, and 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone (**9**) as emodin. Plant polyphenols can be quantified by a calibration curve of structurally related compounds.[37] Calibration curves were constructed with a least-squares linear regression analysis of each representative standard. The regression equation was constructed in the form of $y = ax + b$, where x and y represent the concentration of each compound and the peak area, respectively. Correlation coefficients (r^2) were >0.996, indicating good linearity. Recovery was evaluated as A/B, where A is the peak area obtained for the analyte spiked pre-extraction and B is that obtained for the analyte spiked postextraction. Analyte recovery was determined at three concentration levels of 50, 100, and 200 mg/L. Recoveries were 84.1–104.5%, 85.9–103.1%, and 85.4–102.9% at 50, 100, and 200 mg/L, respectively. Precision was estimated by the relative standard deviation (RSD). The RSD values were <1.9%, 0.3%, and 0.7% at the three concentration levels, respectively. These results indicate that the present assay method is acceptable. The performance limits were expressed in terms of LOD and LOQ. The LOD and LOQ were evaluated using signal-to-noise ratios of 3 and 10 on the chromatogram, respectively. LODs and LOQs were <0.075 and 0.248 mg/L, respectively, indicating good performance.

The concentrations of the nine polyphenols determined in the chrysanthemum leaves that were grown under different supplemental light qualities are compiled in Table 4. The total amount of polyphenols was higher in leaves grown under red and green light than those under white and blue light. While components **3**, **4**, and **7** were major polyphenols of leaves, **2** and **8** were detected as minor components.

PCA Biplot. PCA was performed to obtain an insight into a more obvious relationship between the LED illumination of the different colors and the polyphenol production in the leaves of chrysanthemum, and the results are presented graphically on PCA biplots.

PCA is one of the most suitable and widely used methods to analyze hidden structures in multivariate systems by reducing a large number of variables to a limited number of PCs. The first PC depicts the greatest part of total variation, and the following PCs successively display smaller parts of the original variance.[47,48] Biplots provide a graphic relationship between both samples and variables in a data matrix. Samples are shown as points, whereas variables are exhibited as linear arrows.

The PCA biplot is shown in Figure 6. As shown in Figure 5, PC1 described 84.38% of the original data and PC2 described 10.24%. Because the experiments were repeated in triplicate under each color light, there were three colored points. The

Table 3. Validation Data of Representative Standards (n = 3)

	recovery ± RSD[a] (%)					
standards	50 mg/L	100 mg/L	200 mg/L	r^2	LOD[b]	LOQ[c]
caffeic acid	84.1 ± 0.1	85.9 ± 0.1	89.7 ± 0.2	0.997	0.074	0.248
luteolin	90.3 ± 0.1	94.4 ± 0.1	92.6 ± 0.2	0.998	0.007	0.017
apigenin	96.0 ± 0.3	92.0 ± 0.3	85.4 ± 0.2	0.999	0.019	0.037
naringin	86.7 ± 0.3	87.4 ± 0.2	95.9 ± 0.7	0.998	0.065	0.217
quercetin	104.5 ± 1.4	103.1 ± 0.2	102.9 ± 0.2	0.996	0.017	0.035
emodin	88.6 ± 1.9	92.0 ± 0.3	97.4 ± 0.3	0.999	0.007	0.017

[a]RSD: relative standard deviation. [b]LOD: limit of detection. [c]LOQ: limit of quantitation.

Table 4. Concentrations of Nine Polyphenols in the Leaves of Chrysanthemum Grown under Different Supplemental Light Qualities

	concentration (mg/kg)			
cmpds[a]	blue	green	red	white
1	15.9 ± 0.7 c	37.9 ± 0.4 a	37.3 ± 1.8 a	23.3 ± 0.2 b
2	4.8 ± 0.5 b	13.2 ± 0.1 a	13.9 ± 1.9 a	5.7 ± 1.3 b
3	71.6 ± 1.0 b	145.1 ± 1.0 a	143.9 ± 2.2 a	55.5 ± 0.7 c
4	88.2 ± 1.9 c	188.6 ± 1.7 a	200.7 ± 4.3 a	147.1 ± 10.5 b
5	26.1 ± 2.6 b	41.0 ± 0.4 a	44.1 ± 4.1 a	40.1 ± 4.5 a
6	21.1 ± 0.2 c	43.5 ± 0.5 a	45.2 ± 0.2 a	34.8 ± 2.2 b
7	72.2 ± 3.9 b	90.7 ± 2.7 a	97.4 ± 5.9 a	89.7 ± 0.8 a
8	10.6 ± 0.5 c	22.1 ± 1.2 a	15.0 ± 1.0 b	11.0 ± 0.8 c
9	19.1 ± 1.1 c	35.9 ± 3.2 a	39.1 ± 0.4 a	24.4 ± 0.1 b
total	329.6 ± 11.4 c	618.0 ± 6.8 a	636.6 ± 21.5 a	431.6 ± 21.0 b

[a]The compounds numbers correspond to those given in Table 2. Data represent the mean ± SD of triplicate determinations by HPLC−UV method. In each raw, different letters indicate a significant difference (Tukey's test, $P < 0.05$).

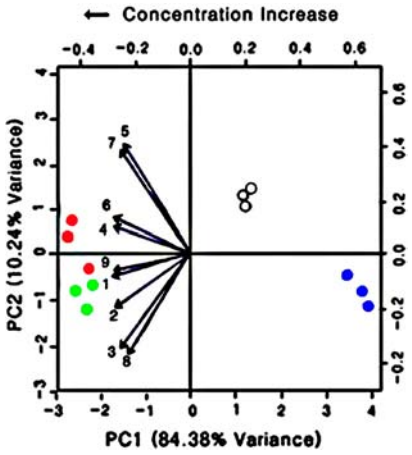

Figure 6. Principal component analysis (PCA) biplot of nine polyphenols characterized for chrysanthemum leaves grown under different supplemental light qualities: red spots, red light illumination; green spots, green light; white spots, white light; blue spots, blue light; (**1**) chlorogenic acid; (**2**) luteolin-7-*O*-glucoside; (**3**) luteolin-7-*O*-glucuronide; (**4**) dicaffeoylquinic acid isomer; (**5**) dicaffeoylquinic acid isomer; (**6**) apigenin-7-*O*-glucuronide; (**7**) naringenin; (**8**) quercetagetin-trimethyl ether; (**9**) 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone.

direction of the arrows represents the increase in each polyphenol concentration. The position that each colored point was projected perpendicularly onto the individual arrow axis signifies the relative concentration of the corresponding polyphenol. Three green and three red points are projected on the leftmost of the arrow axes, which means high production of the polyphenols under the corresponding light illumination, whereas three white and three blue points represent the poorest production. Thus, polyphenols **2**, **3**, and **8** yielded the highest amount under green light, and polyphenols **5**, **7**, **6**, and **4** were greatest under red light. Polyphenols **1** and **9** were produced in similar concentrations under both lights. In contrast, the white and blue light appeared inefficient for polyphenol production.

Light quality might be associated with polyphenol biosynthesis in higher plants. However, the light effect on polyphenol production remains poorly understood. Thus, while red and blue light have been reported to be favorable for polyphenol biosynthesis in some plants,[49,50] white light may be advantageous compared with blue and red light.[51] No study has been carried out to assess the effects of green light on polyphenol production until now.[52] Therefore, further studies should be conducted to better understand the effect of light quality on polyphenol production as well as flowering and organ growth.

In conclusion, flower budding under blue light illumination appeared not to follow the critical photoperiod. While leaf and stem growth was greatest under white light illumination, root growth continued regardless of light quality. Three polyphenols, naringenin (**7**), quercetagetin-trimethyl ether (**8**), and 1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone (**9**), were identified for the first time in chrysanthemum. Quantitative analysis and a PCA biplot clearly demonstrated that red and green light were more effective than white and blue light for polyphenol production.

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Author Contributions

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by a KBSI Grant (T32710) to S.C.S. and J.S.J.

ABBREVIATIONS USED

LEDs, light-emitting diodes; HPLC−MS/MS, liquid chromatography−tandem mass spectrometry; PCA biplot, principal component analysis biplot

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