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Effect of LED Lights on Secondary Metabolites and Antioxidant Activities in Red Pakchoi Baby Leaves

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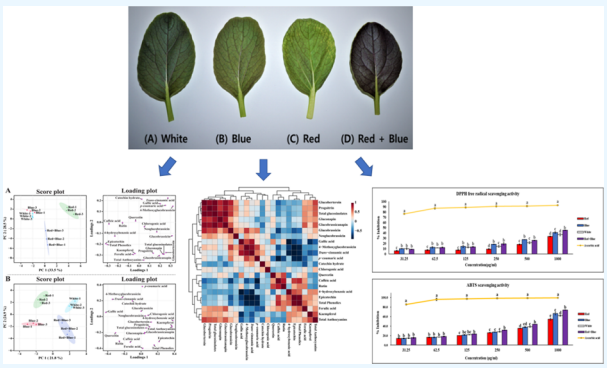
ABSTRACT: Pakchoi (*Brassica rapa* subsp. *chinensis*) is one of the most widely consumed vegetables in Asian countries, and it is high in secondary metabolites. The availability, quantity, and quality of light play a critical role in the growth and development of plants. In this study, we investigated the effect of LEDs (light-emitting diodes; white, blue, red, and red + blue) on anthocyanin, glucosinolates, and phenolic levels in red pakchoi baby leaves. On the 24th day after sowing (DAS), red baby pakchoi leaves were harvested, and shoot length, root length, and fresh weight were measured. Among the different LED treatments, there was no significant difference in shoot length, whereas the highest root length was achieved in the red + blue LED treatment (23.8 cm). The fresh weight also showed a significant difference among the different LED treatments. In total, 12 phenolic and 7 glucosinolate individual compounds were identified using high-performance liquid chromatography (HPLC) analysis. The highest total glucosinolate (2937 μg/g dry wt) and phenolic (1589 μg/g dry wt) contents were achieved in baby leaves exposed to red + blue light. Similarly, the highest contents of total anthocyanins (1726 μg/g dry wt), flavonoids (4920 μg/g dry wt), and phenolics (5900 μg/g dry wt) were achieved in the red + blue treatment. Plants exposed to red + blue LED light showed the highest accumulation of anthocyanin, glucosinolates, and phenolic compounds. For antioxidant activity, DPPH (2,2-diphenyl-1-picrylhydrazylradical) free radical scavenging, ABTS (2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) radical scavenging, and reducing power assays were performed, and the antioxidant activity of red pakchoi baby leaves grown under red + blue LED light was found to be the best. The metabolic profiling of the identified metabolites revealed distinct separation based on the secondary metabolites. This research will be helpful for farmers to choose the best LED light combination to increase the secondary metabolic content in pakchoi plants.



1. INTRODUCTION

Pakchoi, also known as Chinese cabbage (*Brassica rapa* subsp. *chinensis*), belongs to the Brassicaceae family. Because of its beneficial phytochemical composition, it is grown and consumed annually in most Asian countries.[1] It is grown and consumed throughout the year because it has numerous health-promoting compounds, such as anthocyanins, carotenoids, chlorophylls, flavonoids, hydroxycinnamic acid derivatives, isorhamnetin, kaempferol, phenolics, quercetin, vitamins, and minerals.[2,3]

Glucosinolates are a large group of plant secondary metabolites with nutritional effects and biological activity. Glucosinolates are primarily found in cruciferous plants, including the widely consumed Brassicaceae family.[4] One of the reasons for the high consumption of cruciferous vegetables is that the breakdown products of glucosinolates, such as nitriles, oxazolidines, thiocyanates, isothiocyanates, and epithionitriles, are effective in reducing the risk of cancer and heart failure in humans.[5,6] One of the most important groups of phytochemicals with antioxidant capacity is phenolic compounds.[7] The phenolic content of Brassica vegetables has recently been examined. Despite variations among species and even harvests from the same species, these crops are usually rich in phenolic compound composition.[8]

Among the various environmental factors, light quality is crucial for photosynthesis, plant growth, and development.[9] In plants, light-emitting diode (LED) affects metabolites in a significant way and plays an important role in their physiology.[10] Furthermore, LED lights are more effective than fluorescent lights for nonheading pakchoi reproductive and vegetative growth. Blue LED light promotes vegetative growth, whereas red LED light promotes reproductive growth.[11] Several studies have reported that different LEDs

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have a significant effect on growth and secondary metabolite production (anthocyanins, carotenoids, glucosinolates, and phenolics) in various leafy vegetable microgreens, and sprouts were studied such as canola sprout,[6] kale,[10] kohlrabi sprout,[12] lettuce,[13] mustard sprout,[11] and Tartary buckwheat sprout.[14]

Baby leaf vegetables are grown in high densities, harvested, and used when the leaves are young (juvenile stage).[15] The baby leaf market is growing rapidly owing to its convenience and health benefits.[16] The method of growing fresh vegetables with LEDs has shown many positive aspects, such as compact bulb material, long life, specific wavelength, and ease of adjustment.[17] In addition, LED lights have produced antioxidant properties in various crops, such as pea seedlings, spinach, lettuce, and komatsuna.[18] According to Yeo et al., LED arrays increase the accumulation of secondary metabolites including carotenoids, flavonoids, glucosinolates, and phenolic acids.[19]

It is easier and more effective to control and evaluate the effects of light intensity on the biosynthesis of the desired substances.[17] However, in red pakchoi baby leaves, analyses of secondary metabolites after exposure to LED light have not been studied well. Hence, this study aims to provide the optimized light conditions for the plant growth, secondary metabolite production, and antioxidant potentials of pakchoi baby leaves via HPLC analysis of phenolics and glucosinolates and DPPH, ABTS, and reducing power assays.

2. MATERIALS AND METHODS

2.1. Plant Materials. Red pakchoi (*B. rapa* subsp. *chinensis*) seeds were obtained from Asia Seed Co., South Korea. 1.0 g of seed was sterilized with 70% ethanol (v/v), sown in an 11 cm × 11 cm plastic pot with vermiculite soil, and exposed to white (449−551 nm), blue (452 nm), red (636 nm), and red + blue (457 and 636 nm) LED lights with a flux rate of 90 μmol·m^{-2}·s^{-1}. The experiment was arranged in a completely randomized design (CRD) with ten replications. In an LED growth chamber (Multi-Room Chamber HB-302S-4, Hanbaek Scientific Co., 136 cm × 78 cm × 168 cm), the growth conditions were set at 25 °C with an 8 h dark and 16 h light cycle. The red baby pakchoi leaves were collected 24 days after sowing (DAS). For growth measurements, 24 DAS, ten plants from each treatment were selected randomly, and growth measurements were taken. The shoot length (SL) and root length (RL) were measured in centimeters using a meter ruler. To determine the fresh weight (FW), the pakchoi roots were weighed in milligrams using a balance. For HPLC examination, the samples were collected, freeze-dried at −80 °C for 72 h, and then crushed into a fine powder using a mortar and pestle.

2.2. Glucosinolate Extraction and HPLC Analysis. 0.1 mg sample was collected in a 5 mL Eppendorf tube for glucosinolate extraction, and 1.5 mL of 70% (v/v) methanol was added and stirred at 70 °C. Following that, the mixers were placed at 70 °C in a water bath for almost 5 min to inactivate the myrosinase enzyme, after which the sample was centrifuged for 10 min at 12,000 rpm. To obtain 4.5 mL of the supernatant, this procedure was repeated three times. A mini-column containing DEAE-Sephadex A-25 was loaded with the collected supernatant, followed by the addition of 2 mL of water with the purpose of blocking the end of the mini-column and then adding 75 μL of arylsulfatase enzyme solution for desulfation. Then, the mini-columns were left at room temperature for 16 h. For HPLC analyses, desulfoglucosinolates were eluted with 0.5 mL of HPLC-grade water. Subsequently, the prepared solutions were filtered through a 0.45 μm syringe filter. HPLC analysis and conditions were like those in a previous study.[20] The identification and quantification of seven glucosinolates were performed based on our database comparing retention times, and 0.1 mg/mL sinigrin was used as an external standard (Table S1).

2.3. Phenylpropanoid Extraction and HPLC Analysis. A volume of 2 mL of 80% (v/v) methanol was added to 100 mg of pakchoi tissue powder samples. Subsequently, the mixture was vortexed and then placed in a sonicator at 36 °C for 1 h. After sonication, the samples were centrifuged at 12,000 rpm for 10 min at 4 °C to collect the supernatant. Using 0.45 μm Whatman No. 42 filter paper, the supernatant samples were transferred to new tubes and stored in vials for HPLC analysis. HPLC analysis and conditions were like those in a previous study.[20] The retention times of the standard and spike tests were used to specify the peaks in the extract. Also, quantification was performed through each calibration curve of 12 compounds (Table S2). The standard compounds for phenolics analysis are gallic acid (≥99%), caffeic acid (≥98%), 4-hydroxybenzoic acid (=99%), (−)-epicatechin (≥98%), rutin (≥94%), *trans*-cinnamic acid (≥95%), chlorogenic acid (≥95%), ferulic acid (=99%), catechin hydrate (≥98%), *p*-coumaric acid (≥98%), quercetin (≥95%), and kaempferol (≥97%), which were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO).

2.4. Measurement of Total Anthocyanin Content by Spectrophotometry. To determine the total anthocyanin content (TAC), 100 mg of a dry weight sample was collected in a 5 mL tube, and then, 2 mL of 70% ethanol was added and sonicated for 1 h. Then, the sample was centrifuged at 12,000 rpm for 20 min at 4 °C, followed by filtration into an Eppendorf tube through a 0.45 μm PTFE hydrophilic syringe filter. The TAC was quantified using a pH differential method with a two-buffer system: potassium chloride buffer, pH 1.0 (25 mM), and sodium acetate buffer, pH 4.5 (400 mM).[21] To determine the total anthocyanin content, 0.4 mL of the extraction solution was mixed with 3.6 mL of each buffer. The absorbance of each solution was measured using the UV−vis spectrophotometer SPECTRO star nano-plate reader (BMG LABTECH) at 510 and 700 nm against a blank in a cuvette with a 1 cm path length. The total anthocyanin content was calculated as previously described. The final anthocyanin content was expressed as cyanidin-3-glucoside equivalent (CYE) in 1 g of dried powder (mg CYE/1 g dried powder). For each sample extraction, the anthocyanin content was determined in triplicate.

2.5. Total Phenolic and Total Flavonoid Content (TPC and TFC). 100 mg of the dried sample powder was mixed with 2 mL of methanol (70%, v/v), followed by sonication for 60 min at 25 °C. The mixture was centrifuged for 20 min at 12,000 rpm and 4 °C, and then, the supernatant was filtered through a 0.45 μm PTFE hydrophilic syringe filter. The TPC and TFC were measured using the protocol described by Kumla et al. and Park et al.[22,23] Sample extracts were diluted with methanol (70%, v/v) to a concentration of 5000 ppm. For the TPC analysis, 0.5 mL of 2 N Folin and Ciocalteu phenol reagent (Sigma-Aldrich Co., Yongin, Korea) was mixed with 0.1 mL of each extract and incubated at room temperature for 3 min. Afterward, 4 mL of 20% (v/v) Na_2CO_3 solution was added to the mixture and then incubated in the dark for 90 min. The absorbances of each sample were measured at 760

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nm using the SPECTRO star Nano-plate reader (BMG LABTECH). The total phenolic contents were quantified by using the equivalent calibration curve of gallic acid (ranging from 0 to 493.75 μg/mL; $y = 0.0012x + 0.0558$, $R^2 = 0.997$). For the TFC analysis, 0.5 mL of diluted extracts was mixed with 2.0 mL of distilled water. Thereafter, 0.15 mL of 5% (v/v) $NaNO_2$ was added to the mixture, followed by incubation for 5 min at 25 °C. A 0.15 mL portion of 10% (v/v) $AlCl_3$ was added and then maintained for 15 min at room temperature. The absorbances of each sample were measured at 415 nm. The flavonoid contents were quantified by using the equivalent calibration curve of quercetin (ranging from 0 to 1000 μg/mL; $y = 0.0022x + 0.0371$, $R^2 = 0.998$). All of the results are presented as the average of three replications and expressed in terms of milligrams of gallic acid or quercetin equivalent per gram of dry weight (mg gallic acid or quercetin equivalent (GAE or QE)/g DW).

2.6. Antioxidant Assays. *2.6.1. DPPH Free Radical Scavenging Assay.* The DPPH free radical scavenging activity was assessed as previously described.[24] 100 μL of each sample extract with concentrations ranging from 31.25 to 1000 μg/mL was placed in 96-well plates, and then, 100 μL of a 0.2 mM DPPH solution was added. The mixtures were left in the dark for 30 min and then measured at 517 nm using the UV−vis spectrophotometer. The DPPH radical scavenging activity was calculated with the following formula: DPPH radical scavenging activity (%) = $[1 - \{(A_0 - A_1)/A_2\}] \times 100$, where A_0 is the sample absorbance, A_1 is the blank absorbance, and A_2 is the control absorbance.

2.6.2. ABTS Radical Scavenging Assay. The ABTS radical scavenging activity was assessed as previously described.[25] A 7 mM concentration of ABTS powder was dissolved in a 2.5 mM potassium persulfate solution and then incubated for 16 h in the dark. The absorbance of the ABTS buffer solution was adjusted to 0.7 ± 0.002 at 734 nm using distilled water. 50 μL of each sample extract with concentrations ranging from 31.25 to 1000 μg/mL was placed in 96-well plates, and then, 150 μL of the ABTS buffer solution was added. The mixture was incubated in the dark for 10 min and then measured at 517 nm using a UV−vis spectrophotometer. The ABTS radical scavenging activity was calculated with the following formula: ABTS radical scavenging activity (%) = $\{A_0 - (\text{sample} - \text{blank})\}/A_0 \times 100$, where A_0 is the absorbance of ABTS mixed with methanol.

2.6.3. Reducing Power Assay. The reducing power assay was performed as previously described.[26] 300 μL of each sample extract with concentrations ranging from 31.25 to 1000 μg/mL was mixed with 300 μL of 0.2 M phosphate buffer, followed by 300 μL of 1% (v/v) $C_6N_6FeK_3$ solution. After incubation for 20 min at 50 °C, 300 μL of 10% (v/v) $C_2HCl_3O_2$ solution was added and then vortexed. The mixtures were centrifuged at 10,000 rpm for 10 min, and 500 μL of supernatants was mixed with 500 μL of distilled water, followed by the addition of 100 μL of 0.1% (v/v) $FeCl_3$ solution. The solution was measured by using a UV−vis spectrophotometer at 700 nm. An increase in absorption value at this wavelength indicates a high reducing power.

2.7. Statistical Analysis. The shoot and root length, fresh weight, anthocyanin, flavonoid, phenolics, antioxidant activities, and HPLC data were analyzed using Tukey's multiple range test using R at $p < 0.05$.[27] For metabolic profiling, PCA (principal component analysis), PLS-DA (partial least-squares discriminant analysis), heatmap, Pearson correlation analysis, and VIP (variable importance in projection) were performed using MetaboAnalyst 5.0 (http://www.metaboanalyst.ca/, accessed on June 9, 2023) with autoscaling. Heatmap is used to visualize numerical values of phenolics and glucosinolates.

3. RESULTS

3.1. Shoot Length, Root Length, and Fresh Weight of Pakchoi Plantlets. On the 24th day after sowing, shoot length, root length, and fresh weight were measured from four different LED light-exposed pakchoi plantlets (Figure 1). The

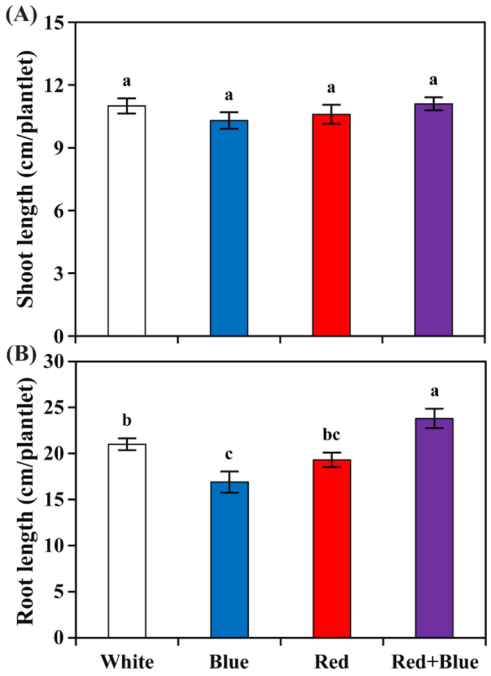

Figure 1. (A, B) Effect of different LED lights on shoot length and root length in pakchoi baby leaves was determined 24 DAS in growth chambers. Different letters denote a significant difference in means ($p < 0.05$) using the Tukey test.

results showed that there was no statistically significant effect on the shoot length. However, the root length of the pakchoi plantlet showed a significant difference among the different types of LED exposures, with the longest length in the red + blue LED light (23.80 ± 1.05 cm) and the shortest length in the blue LED light (16.90 ± 1.15 cm). Like the shoot length, the fresh weight also showed a statistically significant difference among the different LED-exposed plantlets (Figure 2). From these, it can be seen that the exposure of red pakchoi baby leaf to different LED lights did not show any significant difference in shoot length, whereas the root length and fresh weight showed a significant difference among the different LED treatments. However, the highest shoot length (11.10 ± 0.31 cm), root length (23.80 ± 1.05 cm), and fresh weight (5.09 ± 0.29 g) were observed in the red + blue treatment.

3.2. Glucosinolate Accumulation in Red Pakchoi Baby Leaves. Regarding the glucosinolate content, a total of seven glucosinolates, composed of four aliphatic glucosinolates (glucobrassicanapin, gluconapin, glucoberteroin, and progoitrin) and three indolic glucosinolates (4-methoxyglucobrassicin, glucobrassicin, and neoglucobrassicin), were identified. In general, the concentrations of total glucosinolates in red pakchoi baby leaves grown under blue and white LED lights

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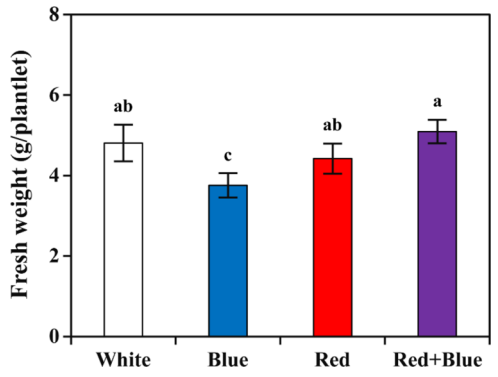

Figure 2. Effect of different LED lights on fresh weight in pakchoi baby leaves was determined 24 DAS in growth chambers. Different letters denote a significant difference in means ($p < 0.05$) using the Tukey test.

presented no statistically significant difference (2262 ± 148 and 2228 ± 64 μg/g dry wt, respectively). However, the plants grown under red and red + blue LED lights showed the highest glucosinolate concentrations (2688 ± 148 and 2938 ± 217 μg/g dry wt, respectively). Regarding the individual glucosinolate concentrations, high concentrations of progoitrin, followed by gluconapin and glucobrassicanapin, were found in pakchoi plants grown under red + blue LED light, whereas in red LED light, the highest contents were found for glucobrassicin and 4-methoxyglucobrassicin. Moreover, higher levels of neoglucobrassicin were found in plants exposed to red and red + blue LED lights (Table 1).

3.3. Phenolic Compounds in Red Pakchoi Baby Leaves. Twelve individual phenolic compounds, comprising five hydroxycinnamic acids (caffeic acid, ferulic acid, *p*-coumaric acid, chlorogenic acid, and *trans*-cinnamic acid), two hydroxybenzoic acids (gallic acid and 4-hydroxybenzoic acid), three flavonols (quercetin, rutin, and kaempferol), and two flavan-3-ols (catechin hydrate and epicatechin), were identified. The highest concentration of phenolic compounds was acquired in plants exposed to red + blue LED light (1589 ± 24 μg/g dry wt); however, no statistically significant difference was observed with plants exposed to blue and white LED lights. The lowest concentrations were obtained in plants exposed to red LED light (1340 ± 11 μg/g dry wt). Regarding each phenolic compound, gallic acid, catechin hydrate, *p*-coumaric acid, and *trans*-cinnamic acid were higher in plants exposed to red LED light. For plants grown under red + blue LED light, the following compounds had higher concentrations: chlorogenic acid, epicatechin, ferulic acid, and kaempferol, whereas in the plants exposed to blue LED light, caffeic acid, rutin, and quercetin were the highest. In the white LED light, only one compound (4-hydroxybenzoic acid) showed the highest accumulation (Table 2). From this result, it can be seen that baby leaves exposed to blue, white, and red + blue LED do not show any significant difference in the phenolic content; however, the highest phenolic content was observed in the red + blue LED light-exposed leaves.

3.4. Total Anthocyanin, Flavonoid, and Phenolic Compounds. The highest total anthocyanin content was detected under the red + blue LED light treatment (1727 ± 29 μg of CYE/g dry wt), and the lower content was detected under blue LED light (708 ± 97 μg of CYE/g dry wt). There was a statistical difference between all LED light treatments (Table 3). The white, red, and blue light treatments showed a lower anthocyanin content compared with the red + blue LED light treatment. The highest total flavonoid content was detected under the red + blue LED light treatment (4920 ± 140 μg of QE/g dry wt), and the lowest content was detected under red LED light (2910 ± 90 μg of QE/g dry wt.). There was a statistical difference between LED light treatments (Table 3). Compared to blue and white LED light treatment, the total flavonoid concentration is higher in red + blue light, but the concentration is lower in red LED light treatment. Likewise, the highest total phenolic content was detected under the red + blue LED light treatment (5900 ± 90 μg of GAE/g of dry wt), and the lowest content was detected under white LED light (3480 ± 190 μg of GAE/g of dry wt). These findings showed that red + blue LED light exposure significantly accumulates the total anthocyanin, flavonoid, and phenolic content in the red pakchoi baby leaves.

3.5. Metabolic Profiling of Identified Compounds. Seven individual glucosinolates, 12 phenolic compounds, and total anthocyanin were identified and quantified from the pakchoi baby leaves exposed to different carbon sources. The heatmap was mainly grouped into two clusters, namely, clusters 1 and 2. Cluster 1 was further subdivided into two subgroups, 1a and 1b, and cluster 2 was also subdivided into clusters 2a and 2b (Figure 3). Cluster 1 consisted of the compounds highly present in the red + blue, white, and blue LED treatments, whereas cluster 2 was separated based on the compounds highly present in the red and red + blue LED light treatments. Cluster 1a formed a separation based on the highest amounts of phenolic compounds (ferulic acid, quercetin, caffeic acid, and rutin) present in the red + blue and blue LED light treatments, whereas cluster 1b was grouped based on the phenolic compounds (kaempferol, 4-hydroxybenzoic acid, epicatechin, and total phenolics) and total

Table 1. Glucosinolate Concentrations (μg/g Dry wt) in Red Pakchoi Baby Leaves Grown under Different LED Lights[a]

	LED light (μg/g dry wt)			
glucosinolates	red	blue	white	red + blue
progoitrin	588.46 ± 38.46^{a}	469.37 ± 45.90^{b}	472.22 ± 39.35^{b}	661.88 ± 58.24^{a}
gluconapin	812.30 ± 64.91^{ab}	771.63 ± 44.29^{ab}	738.83 ± 43.37^{b}	890.62 ± 110.41^{a}
glucobrassicanapin	487.88 ± 53.04^{b}	409.92 ± 53.54^{c}	373.89 ± 21.14^{c}	591.53 ± 25.54^{a}
glucobrassicin	247.27 ± 8.14^{a}	196.95 ± 7.30^{b}	201.56 ± 13.38^{b}	235.01 ± 17.37^{a}
4-methoxyglucobrassicin	67.10 ± 3.01^{a}	41.2 ± 3.88^{b}	36.50 ± 6.15^{b}	39.88 ± 0.87^{b}
glucoberteroin	374.48 ± 35.87^{a}	269.49 ± 19.79^{b}	296.74 ± 14.05^{b}	410.01 ± 26.21^{a}
neoglucobrassicin	110.58 ± 10.69^{a}	103.29 ± 5.82^{a}	104.12 ± 3.06^{a}	108.89 ± 7.29^{a}
total	2688.06 ± 147.82^{a}	2261.90 ± 147.68^{b}	2227.86 ± 63.88^{b}	2937.81 ± 216.65^{a}

[a]Using the Tukey test, different letters denote a significant difference in means ($p < 0.05$).

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Table 2. Concentration of Phenolic Compounds (μg/g Dry Weight) in Red Pakchoi Baby Leaves Grown under Different LED Lights[a]

	LED light (μg/g dry wt)			
phenolic compounds	red	blue	white	red + blue
gallic acid	19.65 ± 0.98^{a}	18.13 ± 1.45^{a}	11.78 ± 0.25^{c}	14.63 ± 1.38^{b}
4-hydroxybenzoic acid	185.45 ± 0.41^{c}	199.83 ± 17.71^{bc}	234.19 ± 1.99^{a}	217.89 ± 10.07^{ab}
catechin hydrate	35.22 ± 0.65^{c}	34.71 ± 0.98^{a}	34.93 ± 0.78^{a}	33.55 ± 1.36^{a}
chlorogenic acid	54.83 ± 1.91^{a}	52.43 ± 5.54^{a}	53.44 ± 2.39^{a}	56.13 ± 6.35^{a}
caffeic acid	95.00 ± 2.35^{c}	143.45 ± 4.90^{a}	112.23 ± 1.49^{b}	118.05 ± 7.08^{b}
epicatechin	354.55 ± 6.18^{b}	507.03 ± 17.03^{a}	510.03 ± 17.12^{a}	520.56 ± 30.02^{a}
p-coumaric acid	115.35 ± 4.75^{a}	89.79 ± 2.11^{c}	103.81 ± 3.26^{ab}	95.05 ± 10.61^{bc}
ferulic acid	125.77 ± 0.27^{c}	143.04 ± 3.48^{b}	122.25 ± 1.18^{c}	160.23 ± 3.19^{a}
rutin	100.65 ± 9.50^{b}	131.29 ± 4.81^{a}	98.83 ± 6.62^{b}	111.84 ± 5.20^{b}
trans-cinnamic acid	14.67 ± 0.77^{a}	9.13 ± 0.37^{b}	5.21 ± 0.43^{c}	5.63 ± 0.66^{c}
quercetin	131.36 ± 9.47^{ab}	141.83 ± 2.80^{a}	123.87 ± 11.33^{b}	131.87 ± 6.17^{ab}
kaempferol	107.72 ± 2.06^{ab}	103.75 ± 3.25^{b}	121.44 ± 8.25^{a}	123.76 ± 15.37^{a}
total	1340.23 ± 10.92^{b}	1574.40 ± 53.18^{a}	1532.01 ± 32.15^{a}	1589.19 ± 23.51^{a}

[a]Using the Tukey test, different letters denote a significant difference in means ($p < 0.05$).

Table 3. Total Flavonoid, Phenolic, and Anthocyanin Contents in Red Pakchoi Baby Leaves Grown under Different LEDL[a]

LED light type	total flavonoid (μg GAE/g DW)	total phenolic (μg QE/g DW)	total anthocyanin (μg/g DW)
red	2905.39 ± 85.05^{c}	4447.45 ± 93.16^{b}	1016.41 ± 76.18^{c}
blue	3957.80 ± 0.00^{b}	4716.39 ± 581.80^{b}	708.03 ± 96.62^{d}
white	3687.18 ± 147.31^{b}	3479.25 ± 186.33^{c}	1225.70 ± 27.34^{b}
red + blue	4920.00 ± 137.79^{a}	5899.72 ± 93.16^{a}	1726.67 ± 29.12^{a}

[a]Using the Tukey test, different letters denote a significant difference in means ($p < 0.05$).

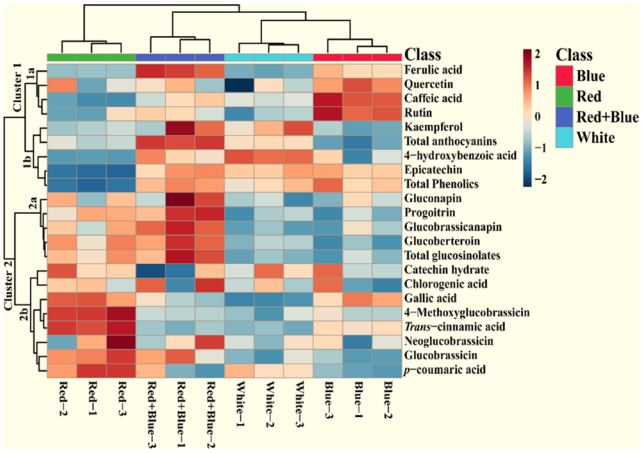

Figure 3. Heatmap with the Euclidean distance measure of relative contents of metabolites in pakchoi baby leaves grown under different LED lights. The relative metabolite concentrations are represented by the color scale (2 to −2) on the right, with high and low concentrations denoted by red and blue, respectively.

anthocyanin compounds rich in red + blue and white treatments. Cluster 2a consisted only of glucosinolate compounds (gluconapin, progoitrin, glucobrassicanapin, glucoberteroin, and total glucosinolates), and they were abundantly present in the red and red + blue LED light treatments, whereas 2b was grouped based on the compounds rich in red treatments, which consisted of both phenolic (gallic acid, *trans*-cinnamic acid, and *p*-coumaric acid) and glucosinolate (4-methoxyglucobrassicin, neoglucobrassicin, and glucobrassicin) compounds. Considering these results, the different LEDs enhanced the accumulation of different metabolites in the red baby pakchoi leaves.

According to the principal component analysis (PCA), PC1 and PC2 showed 33.5 and 28.5% variance, respectively (Figure 4A). In addition, partial least-squares discriminant analysis (PLS-DA) was carried out to exploit the separation between the LED-treated platelets. This clear separation might be due to the gallic acid, *trans*-cinnamic acid, quercetin, rutin, and 4-methoxyglucobrassicin, as well as the associated eigenvector values (Figure 4B). Additionally, the most crucial metabolites of variable importance in projection (VIP) analysis for the prediction were gallic acid, 4-hydroxybenzoic acid, kaempferol, rutin, and total anthocyanin, which led to a great separation (Figure 5). This supports the heatmap results, which showed that the different LEDs enhanced the accumulation of different metabolites in red baby pakchoi leaves.

3.6. In Vitro Antioxidant Assays. The samples showed a concentration-dependent increasing pattern. In particular, mixed (red + blue) LED light has the highest DPPH radical scavenging activity (45.31 ± 0.50%) at 1000 μL/mL concentrations, followed by blue LED (40.89 ± 4.65%) and white LED (36.74 ± 2.13%) treatments (Figure 6A). On the other hand, red LED treatment has the lowest radical scavenging activity (33.28 ± 1.65%) at 1000 μL/mL concentrations. Similarly, the baby leaves revealed concentration-dependent scavenging of ABTS (Figure 6B). The mixed LED-treated baby leaf extracts at 125, 250, 500, and 1000 μL/mL concentrations showed the highest ABTS scavenging activity compared with those of extracts under the other LED light conditions. However, the red LED-treated baby leaf extracts at the same concentrations showed the lowest scavenging activity. Similarly, the reducing power assay showed that the baby leaves under the mixed LED treatment had the best antioxidant ability (Table 4).

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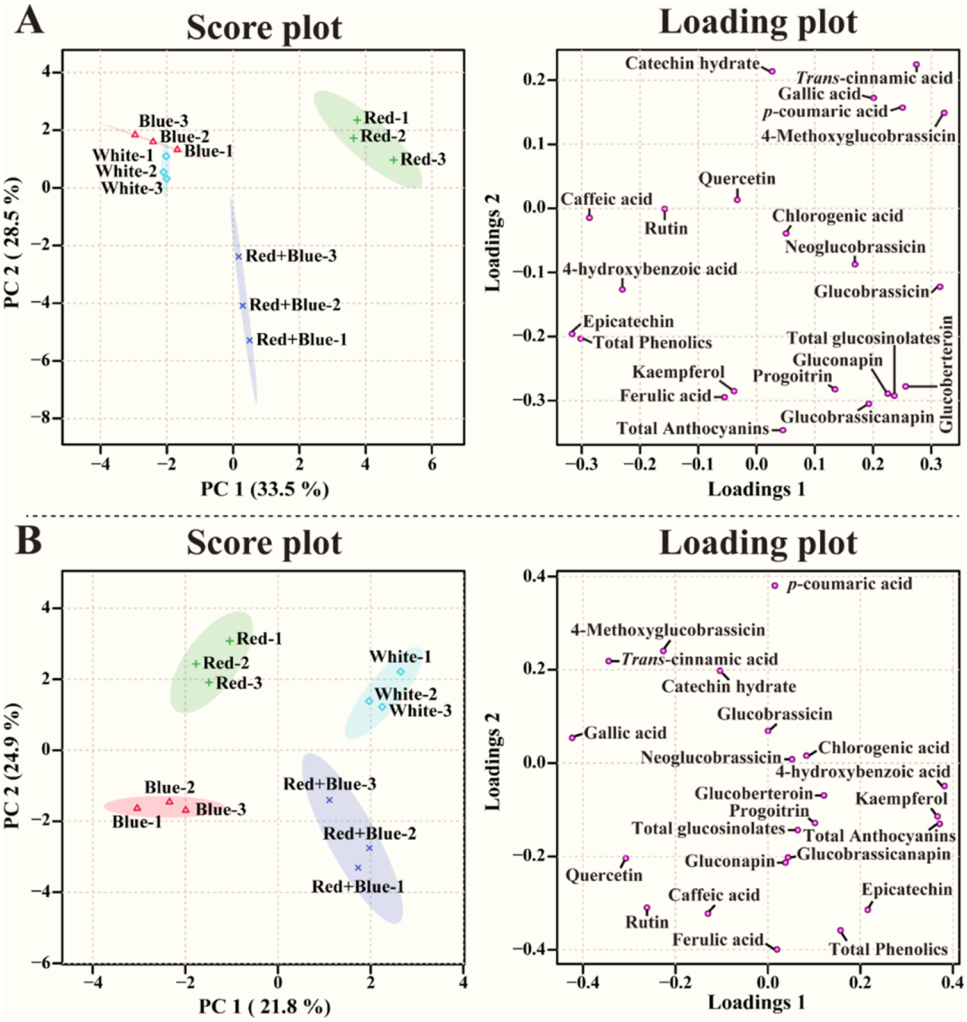

Figure 4. PCA (A) and PLS-DA (B) scores and loading plots of the metabolites found in pakchoi leaves grown under different LED lights.

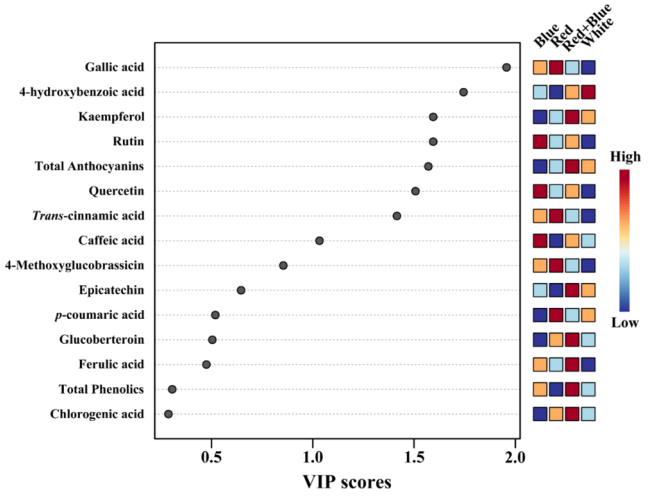

Figure 5. Main components separating pakchoi leaves grown under different LED lights are based on the VIP scores attained via the PLS-DA model. The VIP scores are shown by the black dots, reflecting the degree of importance of the metabolites.

4. DISCUSSION

Light is very important for plant growth and morphology.[10,28] Plant growth and physiological characteristics are greatly influenced by light effects.[29,30] In the current study, in terms of shoot length and fresh weight, the exposure of plants to different LED lights did not affect these variables; however, regarding root length, plants exposed to red + blue LED light obtained the highest values (23.80 ± 1.05 cm). These results are consistent with a previous study on canola (*B. napus* subsp. *napus*), which showed that the highest root length was achieved in red + blue LED light compared to other light treatments; this demonstrates that LED light is a valuable source for crop development.[31] However, different results with the same crop were found; for instance, it has been reported that the highest shoot length and fresh weight were obtained in canola sprouts exposed to red LED light, and for root length, no difference was found between the different LED lights.[6] However, after a 60-day cultivation period of sarcandrae (*Sarcandra glabra*) seedlings, the fresh weight was significantly higher in the white and blue LED light treatments than in the red LED light treatment. Moreover, treatments under blue LED light resulted in considerably longer roots than under red and white LED lights.[32] Buckwheat (*Fagopyrum tataricum*) sprouts were exposed to different LED lights for 10 days. The highest lengths and fresh weights were achieved under red LED light, whereas the lowest values were obtained after blue LED light exposure.[33] When grown under blue LED light, cowpea (*Vigna unguiculata*) sprouts had 1.40 and 1.17 times

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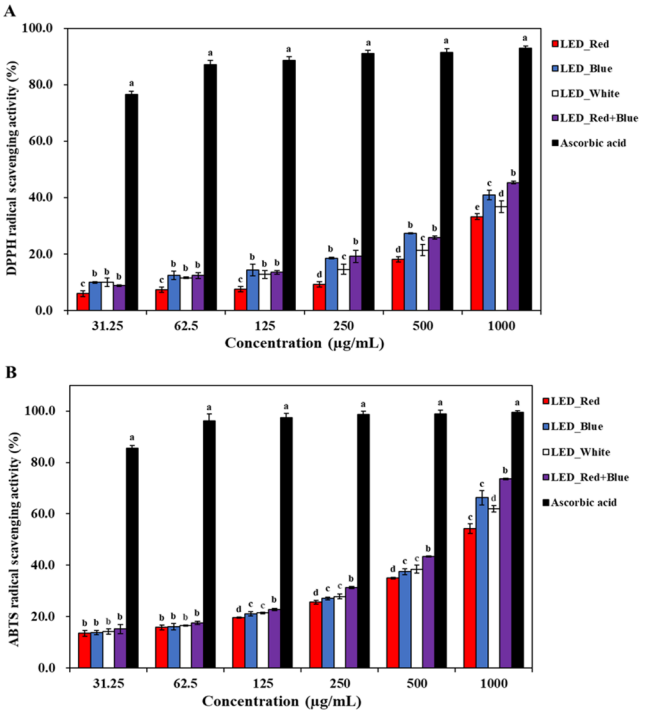

Figure 6. (A,B) DPPH and ABTS free radical scavenging activity in red pakchoi baby leaves grown under different LED lights. Different letters in the values denote statistically significant differences among the means, using the Tukey test ($p < 0.05$), while values are shown as mean ± SD.

higher shoot length and fresh weight, respectively, than those grown under white LED light.[17] Therefore, these results showed that different LED lights have different effects on different *Brassica* species. In the present study, each plant had a different response to different types of LED light irradiation. In pakchoi (*B. rapa* subsp. *chinensis*), exposure to different types of LED light had no significant effect on shoot length; however, red + blue LED light irradiation affected the root length and fresh weight.

In the current study, PCA analysis showed that the red + blue LED group was isolated from the other groups, and it was due to the higher levels of glucosinolates and phenolics. Thus, the combination of red and blue LEDs enhanced the production of these secondary metabolites.[18,19,34] In our study, the highest glucosinolate levels were found in the leaves exposed to red and red + blue LED lights (2937.81 ± 216.65 μg/g dw). Moreover, pretreatment of two *Melissa officinalis* genotype seedlings with different LED lights showed that the red + blue LED light enhances the accumulation of total phenolic and anthocyanin content.[35] It was observed that red and blue LED lights produced noticeably high glucosinolate content in *B. rapa* subsp. *pekinensis* and *Brassica oleracea* var. *acephala*.[36] In addition, they found that glucobrassicanapin and sinigrin were the predominant glucosinolates in *B. rapa* subsp. *pekinensis* and *B. oleracea* var. *acephala*, and also that, compared to dark light, different light conditions increased the amounts of glucosinolates in broccoli sprouts.[37] However, this indicated that there was no statistically significant difference in the total glucosinolate content of *B. napus* sprouts cultivated under white, blue, and red LED lights.[6] The glucosinolate concentration in *B. oleracea* var. *acephala* did not differ significantly between the treatments using red + blue LEDs and white LED light.[38] Similar results were obtained, with no significant difference in the total amount of glucosinolates in the *B. rapa* subsp. *chinensis* var. *parachinensis* sprout grown under white and red + blue LED light; moreover, the amount of glucosinolates gradually decreased with increasing incubation time.[5] Moreover, in this study, no significant difference in the total glucosinolate accumulation was found in red pakchoi baby leaves exposed to blue, white, and red + blue lights. A similar result was found in kohlrabi sprouts exposed to different LED lights, where no significant difference was observed among the different LED exposures.[12] According to previous studies, plant species, a combination of light sources, and the light intensity and spectrum may influence glucosinolate accumulation. In addition, several studies have reported that different *Brassica* cultivars and species accumulate different levels and profiles of glucosinolates and their hydrolysis products based on their genotypes.[39−41]

In the present study, 12 phenolic compounds were identified and measured, and the highest concentration was achieved in plantlets grown under red + blue LED light (1589.19 ± 23.51 μg/g of dw). Furthermore, the highest individual phenolic compound was epicatechin (520.56 ± 30.02 μg/g of dw), which accumulated under red + blue LED light, followed by 4-hydroxybenzoic acid under white (510.03 ± 17.12 μg/g of dw) and blue (507.03 ± 17.03 μg/g of dw) LED lights. Our results differ from those of Kim et al., who found that in *B. rapa* subsp. *pekinensis*, *p*-hydroxybenzoic acid was the most abundant phenolic compound quantified under blue LED light, followed by chlorogenic acid under white LED light.[42] The highest phenolic compound level content of catechin was observed in cowpea sprouts under blue LED light.[17] However, cowpea sprouts grown under white LED light presented the lowest level of phenolic compounds, whereas other LED lights did not affect the content of other phenolic compounds, such as 4-hydroxybenzoic acid and chlorogenic acid.[17] In contrast, the highest rutin content was obtained in buckwheat sprouts grown under blue LED light, whereas no significant difference was observed in the accumulation of chlorogenic acid between sprouts grown under other LED light treatments.[33] In

Table 4. Reducing Power Assay of Red Pakchoi Baby Leaves Grown under Different LED Lights[a]

	absorbance in 700 nm					
	31.25	62.5	125	250	500	1000
ascorbic acid	0.28 ± 0.00^{a}	0.49 ± 0.01^{a}	0.91 ± 0.02^{a}	1.67 ± 0.02^{a}	2.28 ± 0.02^{a}	2.31 ± 0.00^{a}
red	0.08 ± 0.00^{c}	0.09 ± 0.00^{b}	0.09 ± 0.00^{c}	0.11 ± 0.00^{c}	0.13 ± 0.00^{c}	0.19 ± 0.00^{e}
blue	0.09 ± 0.00^{b}	0.09 ± 0.00^{b}	0.09 ± 0.00^{bc}	0.11 ± 0.00^{c}	0.14 ± 0.00^{c}	0.22 ± 0.00^{d}
white	0.09 ± 0.00^{bc}	0.09 ± 0.00^{b}	0.11 ± 0.00^{bc}	0.14 ± 0.00^{b}	0.20 ± 0.00^{b}	0.32 ± 0.00^{c}
red + blue	0.08 ± 0.00^{bc}	0.09 ± 0.00^{b}	0.11 ± 0.00^{b}	0.15 ± 0.00^{b}	0.22 ± 0.00^{b}	0.37 ± 0.00^{b}

[a]Using the Tukey test, different letters denote a significant difference in means ($p < 0.05$).

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Agastache rugosa seedlings, white LED light has been demonstrated to promote the accumulation of phenolics.[43] Moreover, red, blue, or red + blue LED light had no impact on phenolic compound accumulation in tartary buckwheat sprouts.[44] Additionally, fluorescent light exposure resulted in higher concentrations of ginsenoside-Rb1 and ginsenoside-Rg1.[45] In addition, *Aronia melanocarpa*, *Aronia punifolia*, and *Aronia arbutifolia* under blue LED light increased phenolic acid production in their shoots.[46] Similarly, chlorogenic acid was found in the callus of *Peucedanum japonicum*,[47] and a high total phenolic content was found in the callus of *Ocimum bassilicum*.[48] Blue light treatment resulted in the highest accumulation of phenolic compounds in kohlrabi sprouts.[12] However, in this study, red + blue light exposure showed the highest phenolic accumulation. These results show that different LED lights have different effects on phenolic compounds, based on the plant genus.

Anthocyanin can change both quantitatively and qualitatively depending on the lighting conditions.[49] In the present study, we found color changes in leaves exposed to red + blue LED light (1726.67 ± 29.12 μg/g dw), which in turn had the highest anthocyanin concentration. This result is in line with studies that observed that almost 90% of the purple, blue, orange, and red light was absorbed by plants.[50] In a study using three different types of LED lights (white, blue, and red), it was found that the total anthocyanin content in *B. juncea* seedlings varied according to the duration of LED irradiation.[51] The study found that the seedlings had the highest total anthocyanin content after 3 weeks of treatment with blue LED light, followed by red LED light treatment. In contrast, blue LED light enhances the accumulation of anthocyanin compared with white, red, or red + blue LED light in *Fagopyrum tataricum* sprouts.[33] In this study, the total flavonoid and phenolic contents varied depending on the LED conditions. In particular, the highest levels of the total flavonoid (4920.00 ± 137.79 μg GAE/g dw) and phenolic (5899.72 ± 93.16 μg QE/g dw) contents were obtained in the baby leaves under mixed (red + blue) LED light. These findings were consistent with those from previous studies. Jarerat et al. conducted the measurement of TPC and TFC contents accumulated in the peel and flesh of eggplants exposed to various types of LEDs (red, blue, and red + blue), indicating that mixed LED (red + blue) was found to be effective in the accumulation of total phenolics and total flavonoids in the flesh and peel of eggplants.[52] Furthermore, Gam et al. reported that mixed (blue + red) LEDs accumulated the highest total flavone content in *Anoectochilus roxburghii* compared to other light conditions (fluorescent lamp, blue-red, blue:red:white ratios of 1:5:1 and 1:4:2).[53]

In this study, red pakchoi baby leaves exposed to mixed LED (red + blue) had the highest levels of most phenolic compounds, followed by baby leaves under blue LED. It might be due to the activation of the HY5 transcription factor by blue LED lights. HY5 is known to regulate flavonoid biosynthesis under visible light. Particularly, HY5 transcription can be activated by blue light and activates the expression of MYB transcription factors (*MYB12* and *MYB75*) involved in flavonoid biosynthesis. These MYB factors activate the expression of flavonoid biosynthesis genes and lead to an increase in the production of phenolic compounds.[51] Hence, mixed LED (red + blue) and blue LED are considered optimal lights for the production of phenolics in red pakchoi baby leaves.

Plant antioxidant activities are influenced by light conditions. In this study, the antioxidant ability of red pakchoi baby leaves exposed to four different LED light conditions was determined using three different antioxidant assays. The DPPH and ABTS scavenging activities of red pakchoi baby leaves grown under blue and mixed LEDs were relatively higher than those of baby leaves grown under white and red LEDs. The higher antioxidant activities were due to the higher levels of phenolics, anthocyanins, flavonoids, and glucosinolates. The findings were consistent with a previous study reporting that blue LED light increased the accumulation of anthocyanins and phenolic acids in *Brassica juncea* sprouts, and their extracts showed a higher antioxidant capacity.[51] In addition, Jarerat et al. reported that the peel of eggplant exposed to red + blue LED lights had the highest DPPH inhibition and ferric-reducing antioxidant power.[52] As a result, for the accumulation of secondary metabolites (glucosinolates, flavonoids, and phenolic compounds) and antioxidant activity, plants exposed to a combination of red + blue LED light proved to be the most effective.

5. CONCLUSIONS

In the present study, each plant had a different response to different types of LED light irradiations. In pakchoi, exposure to different types of LED lights had no significant effect on shoot length; however, red + blue LED light irradiation affected the root length and fresh weight. Regarding the concentrations of glucosinolates, flavonoids, phenolics, anthocyanin, and antioxidant activity, the current results indicate that plants exposed to a combination of red + blue LED light proved to be the most effective artificial light source. Therefore, this study might establish an effective strategy for improving the antioxidants and the content of some phytochemicals, namely, glucosinolates, flavonoids, phenolic, and anthocyanin production. The metabolic profiling results will provide valuable insights into helping us determine whether pakchoi baby leaves exposed to different LED lights contain high levels of phenolics, glucosinolates, anthocyanin, and antioxidants. This information holds significance for assessing their suitability for human consumption. Further, this study suggested that polyphenols and glucosinolates in pakchoi baby leaves under different LED light sources can work synergistically as antioxidants. Further analysis of metabolite relationships may provide an insight into pakchoi's secondary metabolite biosynthesis pathway under different LED illuminations.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10261.

Additional experimental materials and methods; Table S1, retention times of glucosinolates; Tables S2, calibration curve of phenolic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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