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Effects of light-emitting diodes on the morphology and accumulation of glucosinolates, carotenoids and phenolic acids in red kale sprouts

Leonel Tarcisio da Cristina Bungala^{1,2}, Ramaraj Sathasivam¹, Jong Seok Park³, Jae Kwang Kim⁴*, Sang Un Park^{1,5,6}*

¹Department of Crop Science, Chungnam National University, 99 Daehak-ro, Daejeon 34134, Republic of Korea, ²Mozambique Agricultural Research Institute, Central Regional Center, Highway N° 6, P.O. Box 42, Chimoio, Mozambique, ³Department of Horticultural Science, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Korea, ⁴Division of Life Sciences and Convergence Research Center for Insect Vectors, Incheon National University, Incheon 22012, Republic of Korea, ⁵Department of Bio-Al Convergence, Chungnam National University, 99 Daehak-ro, Daejeon 34134, Republic of Korea, ⁶Department of Smart Agriculture Systems, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

ABSTRACT

Kale (*Brassica oleracea* var. *acephala*) has gained popularity as a nutritious and phytochemical-rich vegetable. This study has investigated the effects of three LED treatments (white, blue, and red) on the accumulation of secondary metabolites in kale sprouts. Ten DAS, the kale sprouts were harvested, and growth measurements were measured. Furthermore, selected sprouts were stored at -80 °C for further biochemical analysis, namely, phenolic acids, glucosinolates, and carotenoids. Sprouts irradiated with red LED light showed the best SL, RT, and FW values. For total GSLs, we found that kale sprouts irradiated with white LED lights showed the best results (41.59±0.41 µmol/g DW). Among the aliphatic GSLs, we found that progoitrin presented the best results under blue LED light (17.93±0.49 µmol/g DW), and among the indolic GSLs, glucobrassicin showed the best results under white and red LED light. The highest concentration of total carotenoids was found in kale sprouts under white LED light (1935.13±87.21 µg/g DW). Among the PAs, chlorogenic acid was found in the highest concentration in the treatments under white LED light (45.78±0.73 µg/g DW). In general, kale sprouts irradiated with white LED light showed the best results under red LED light showed the most promise. This research offers a valuable approach to enhancing the phytochemicals found in kale sprouts under white LED light showed high contents of GSLs, carotenoids, and PAs. Regarding morphological characteristics, kale sprouts under red LED light showed the most promise. This research offers a valuable approach to enhancing the phytochemicals found in kale sprouting.

KEYWORDS: Kale sprouts, LED irradiation, Phytochemical composition, Secondary metabolites, HPLC

INTRODUCTION

Kale (*Brassica oleraceae* var. *acephala*) is a cool-season crop from the Brassicas family. Its leaves, rich in vitamins and essential mineral components, are commonly used alone or together for human and animal feed (Life, 2024). In recent years, kale has gained the scientific community's attention due to its nutritional composition (Ware, 2020), and consumer acceptance (Adeyeye *et al.*, 2018). In addition, due to its high content of bioactive compounds such as vitamins (Becerra-Moreno *et al.*, 2014), glucosinolates (Bhandari *et al.*, 2015; Liu *et al.*, 2022), flavonoids (Lännenpää, 2014; Panche *et al.*, 2016), phenolics (Ayaz *et al.*, 2008; Cartea *et al.*, 2011; Bianchi *et al.*, 2024), micro and minerals (Sikora & Bodziarczyk, 2013; Thavarajah *et al.*, 2016). Notwithstanding, kale has been used in traditional medicinal systems to treat several diseases, including diabetes, rheumatism, and hepatic diseases (Raiola *et al.*, 2017; Chen *et al.*, 2018; Šamec *et al.*, 2019; Luang-In *et al.*, 2020).

A sprout is a young plant that has germinated seeds. There are several varieties of them and they are usually eaten raw (Healthline, 2024). Unsurprisingly, sprouts are nutrient-dense and contain beneficial plant compounds despite their low-calorie content. Depending on the variety, they contain different amounts of vitamins and minerals (Chang *et al.*, 2019). Nutrient levels in sprouted grains, legumes, vegetables, and seeds tend to

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*Corresponding authors: Jae Kwang Kim E-mail: kjkpj@inu.ac.kr Sang Un Park E-mail: supark@cnu.ac.kr Bungala et al.

increase. It is also easier for the human body to absorb all the nutrients in sprouts because they contain fewer antinutrients (Singh *et al.*, 2015; Ghumman *et al.*, 2016; Sibian *et al.*, 2017).

Plants produce primary metabolites as well as secondary metabolites. Secondary metabolites are phytochemicals with no direct function at specific points, but they can play critical roles, such as preventing plants from pathogens and herbivore attacks and attracting pollinators (Wani *et al.*, 2022). In addition, many of the secondary metabolites have been used as active compounds to develop drugs, antibiotics, pesticides, and herbicides (Wani *et al.*, 2022). Among the known phytochemicals, we can highlight polyphenols, flavonoids, anthocyanidins, carotenoids, glucosinolates, and fibres. The presence and secretions of these organisms are found naturally in plants (Alternimi *et al.*, 2017).

Glucosinolates are a large group of secondary metabolites found in plants with nutritional effects and biologically active compounds (Favela-González *et al.*, 2020). Brassicaceae, which includes many cruciferous species, are the most abundant sources of glucosinolates (Barba *et al.*, 2016; Prieto *et al.*, 2019). Recent studies have shown the beneficial effects of glucosinolates, including regulatory functions in inflammation (Connolly *et al.*, 2021), plant stress response, antioxidant activities, and antimicrobial properties (Abdel-Massih *et al.*, 2023).

Phenolic compounds are the most abundant plant secondary metabolites and are ubiquitously present in most plants. They play vital roles in plant defences against various biotic and abiotic stresses and contribute to the development of plant colour (Mark *et al.*, 2019; Kumar *et al.*, 2020; Albuquerque *et al.*, 2021). They are known to have antibacterial (Oussaid *et al.*, 2017), antioxidant (Singh *et al.*, 2017), anti-inflammatory (Velmurugan *et al.*, 2018), and anticarcinogenic activities (Wang *et al.*, 2020). Because of that, plant-based foods rich in phenolic compounds are recommended to enhance human health.

A plant's carotenoids can serve a variety of functions and are secreted by both primary and specialised metabolites, respectively, while in green tissues carotenoids are essential for plant survival as primary metabolites (Liang *et al.*, 2018; Sun & Li, 2020). Previous studies have shown that carotenoids play a role in plant defence response (Uarrota *et al.*, 2018), photo protection (Hashimoto *et al.*, 2016), photosynthesis (Chauhan *et al.*, 2023), plant development and signalling (Dickinson *et al.*, 2019; Felemban *et al.*, 2019). Additionally, carotenoids are essential accessory pigments to enhance nutrition (Giuliano, 2017; Zheng *et al.*, 2020) and reduce the risk of various chronic diseases (Eggersdorfer & Wyss, 2018).

Among various environmental factors, light quality is crucial for photosynthesis, plant growth, and development (Hasan *et al.*, 2017; Song *et al.*, 2020). In plants, light emitting diode (LED) affects the metabolites significantly and plays a vital role in their physiology (Lee *et al.*, 2016). Additionally, Lee *et al.* (2023), in a short-term LED treatment, found a potentially effective enhancement accumulation of phytochemicals. Most sprouts can be easily cultivated indoors and contain higher amounts of phytochemicals. As sprouts are generally consumed raw and cooked lightly, there is no thermal degradation of micronutrients through food processing. Thus, it is required to investigate optimal light conditions to enhance the growth and accumulation of phytochemicals in the sprouts. Therefore, this study aims to examine the effects of three LED treatments on the accumulation of different types of secondary metabolites in *Brassica oleracea* L. var. *acephala* sprouts.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Kale seeds were purchased from Asia Seed Co., Ltd (Seoul, Korea). The experiment was arranged in a completely randomised design (CRD) with three replications for each treatment. Sprouts were established by immersing 20 seeds in sterile water for 24 hours before they were placed in vermiculitefilled plastic pots. The sprouts were grown in a growth chamber at 25 °C under irradiation with white (450-660 nm), blue (450 nm), and red (660 nm) LED lighting with a flux rate of 90 µmol m⁻² s⁻¹ and 16 h photoperiod (PGL-PFL series, PARUS LED Co., Cheonan, Korea). The sprouts were harvested ten days after sowing (DAS). For growth measurements, 10 DAS and ten plants from each subspecies were selected randomly, and growth measurements were taken. The shoot length (SL) and root length (RL) were measured in cm using a meter ruler. To determine the fresh weight (FW), the kale sprouts were weighed in mg using a balance. After harvesting, all sprout samples were frozen in liquid nitrogen, stored at -80 °C, and freeze-dried for high-performance liquid chromatography (HPLC). Glucosinolates and phenolic compounds in sprout mixtures were analyzed by HPLC using samples from three independent replications.

Glucosinolates Extraction and HPLC Analysis

Following previously reported procedures by Lee et al. (2023), glucosinolates (GSLs) were extracted with some modifications. An Eppendorf 2 mL tube containing 100mg of the freeze-dried powdered sample was filled with 1.5 mL of MeOH. Water baths at 70 °C were used for 5 minutes to heat the tubes. A collection of supernatants followed centrifugation at 12000 rpm for 10 minutes at 4 °C in a new 5 mL Eppendorf tube. GSL extracts were prepared from residues by combining the supernatants from both extractions. For desulphating the extracts, 75 µL of aryl sulphatase solution was combined with DEAE-Sephadex A-25 on a Mini column. Then, microcentrifuge tubes filled with 2 mL of H₂O were used to elute DS-GSLs with 0.5 mL ultrapure H₂O, and for the elution, three replicates were performed. Furthermore, GLSs were detected and computed by HPLC peak area ratios, retention times, and response factors using desulpho-sinigrin (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) as an external standard.

Phenolic Acids Determination

A previous method described by Lee *et al.* (2023) was utilised to analyse phenolic acids (PAs). Aqueous MeOH was added

to 100 mg of dried sprout powder samples. Sonication was performed for one hour at 25 °C. The supernatant was transferred to a new tube after centrifugation at 10,000 rpm for 10 minutes. A further two extractions removed sludge. PTFE syringe filters were used to filter the supernatant collected after centrifugation for 15 minutes at 10,000 rpm. According to Lee *et al.* (2023), the HPLC analysis system, gradient program, and conditions were based on their study. The retention times and spiking tests were used to calibrate calibration curves.

Extraction and Analysis of Carotenoids

Carotenoids were obtained using the methods outlined in Park et al. (2012) study. First, 10mg of sprout powder was combined with 3 mL of 0.1% (w/v) ascorbic acid dissolved in EtOH. The mixture was then vigorously mixed for 20 sec and placed in an 85 °C water bath for incubation. After 5 min, 120 µL of NaOH with a concentration of 80% (w/v) was introduced, then for 20 sec, the samples were vortexed and incubated in the 85 °C water bath for 10 min. The samples were then put on ice for 5 min, then 1.5 mL of C_6H_{14} , 1.5 mL of distilled H_2O , and 0.1 mL of the internal standard (β -apo-8'-carotenal in EtOH; 25 μ g/mL) were added to each. Each sample's top C₆H₁₄ layer was moved to a fresh tube after a 5 min centrifugation at 1200 rpm and 4 °C. The centrifugation process was carried out once again for re-extraction. After being dried with nitrogen gas, the supernatants were dissolved in 0.25 mL of a MeOH-CH, CL, solution that was 50:50 (v/v) in concentration, provided the specifications for the HPLC analysis, including the condition, system, and gradient program.

Statistical Analysis

SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used with Duncan's multiple range test at p < 0.05 to analyse the data. Standard deviations and mean values are shown. Three repetitions were performed for each experiment.

RESULTS

Phenotype of Kale Sprouts Irradiated with Different LED Lights

Ten DAS sprouts under red LED light presented the best SL, RT, and FW (8.4 cm, 13.01 cm, and 890 mg, respectively). Kale sprouts irradiated with white LED showed the lowest values in this study. A difference in plant development among treatments was observed, and it was clear that the kale sprouts under red LED light presented better phenotypic characteristics than the others, namely leaf development and plant height (Figure 1).

Content of GSLs in Kale Sprouts Irradiated with Different LED Lights

By HPLC analysis, were identified and quantified a total of 16 GLSs, where 11 aliphatic GSLs (glucoiberin, progoitrin, glucoraphanin, glucoalyssin, gluconapoleiferin, gluconapin, glucobrassicanapin, glucoerucin, glucohirsutin, glucoraphasatin, and glucoberteroin), one aromatic GSLs (gluconasturtiin), and four indolic GSLs (4-methoxyglucobrassicin, glucobrassicin, neoglucobrassicin, and 4-hydroxyglucobrassicin) (Table 1). Progoitrin presented the best results by far in GSL content (white LED, $17.31\pm0.12 \mu mol/g DW$, blue LED, $17.93\pm0.49 \mu mol/g$ DW, and red LED, $13.57\pm0.06 \mu mol/g DW$) compared to other detected. For total GSLs, we found that kale sprouts irradiated with white LED lights showed the best results ($41.59\pm0.41 \mu mol/g DW$).

Carotenoid Content in Kale Sprouts Irradiated with Different LED Lights

Statistical analysis results showed differences in all carotenoids detected (Table 2). The highest concentration of total carotenoids was found in kale sprouts under white LED light exposure ($3341.27 \pm 206.96 \ \mu g/g \ DW$). Individually, β -carotene was found in high concentration (white LED, $1935.13 \pm 87.21 \ \mu g/g \ DW$, blue LED, $1809.35 \pm 58.44 \ \mu g/g \ DW$, and red LED, $1678.86 \pm 26.10 \ \mu g/g \ DW$), followed by lutein. According to these results, it is assumed that the kale sprouts irradiated under white and red LED light may be a better source of β -carotene and lutein, respectively.

Phenolic Acid Content in Kale Sprouts Irradiated with Different LED Lights

Three PAs, two hydroxycinnamic acids (chlorogenic acid and ferulic acid), and one hydroxybenzoic acid (benzoic acid) were detected and quantified in the three LED light treatments (Table 3). Among the PAs, chlorogenic acid was found in the highest concentration in the treatments: white LED ($45.78\pm0.73 \ \mu g/g \ DW$), red LED ($42.01\pm0.81 \ \mu g/g \ DW$), and blue LED ($39.63\pm0.56 \ \mu g/g \ DW$). According to these results, chlorogenic acid occupies an essential position in kale sprout phenolic composition. Overall, the kale sprouts treatment irradiated with white LED light presented the best content in Pas compared with other LED light treatments.

DISCUSSION

In plants, light is a powerful abiotic stimulus that influences growth, development, and morphogenesis (Tariq et al., 2014; Adil et al., 2019). In addition to controlling primary and secondary metabolism, light plays a vital role in ensuring optimum plant growth (Samuolienė et al., 2013; Park et al., 2020a). Our results showed that in kale sprouts grown in 10 days submitted to three different LED light irradiations, those under red LED light irradiations presented the best SL, RL, and FW values. Similar results were found by Park et al. (2019), who reported that the highest shoot length and fresh weight were obtained in canola sprouts exposed to red LED light and for root length. Kochetova et al. (2022) also found the best values in seedlings exposed to red LED light. Additionally, according to a study conducted by Manivannan et al. (2015), R. glutinosa exhibited notable growth improvements when exposed to blue or red LED treatments, as opposed to white light. Furthermore, Thwe et al. (2014) in buckwheat sprouts found that the highest

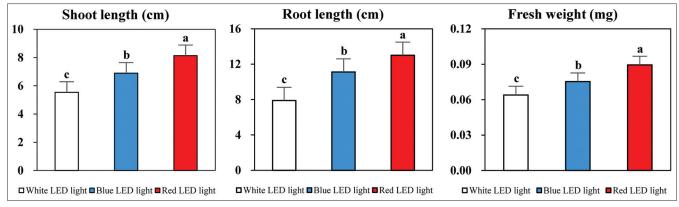


Figure 1: Morphological parameters SL, RT, and FW observed 10 DAS in growth chambers under three different LED light irradiations. Duncan's multiple range test reveals that using different letters signifies a notable distinction in means (p<0.05)

Table 1: The concentration of GSLs (μ mol/g DW) in kale sprouts irradiated with white, blue, and red LED light 10 DAS

Glucosinolates	Concentration (µmol/g DW)			
	White-LED	Blue-LED	Red-LED	
Glucoiberin	$0.36 {\pm} 0.04^{a}$	0.30 ± 0.03^{b}	0.36 ± 0.00^{a}	
Progoitrin	17.31 ± 0.12^{b}	17.93 ± 0.49^{a}	$13.57 \pm 0.06^{\circ}$	
Glucoraphanin	0.89 ± 0.01^{b}	0.97 ± 0.02^{a}	$0.72 \pm 0.02^{\circ}$	
Glucoalyssin	$0.82 {\pm} 0.04^{b}$	0.90 ± 0.01^{a}	$0.68 \pm 0.01^{\circ}$	
Gluconapoleiferin	1.33 ± 0.01^{b}	1.39±0.01°	1.24 ± 0.01^{a}	
Gluconapin	1.69 ± 0.01^{a}	1.53 ± 0.02^{b}	$1.04 \pm 0.01^{\circ}$	
4-Hydroxyglucobrassicin	2.07 ± 0.39^{a}	1.03 ± 0.56^{b}	$1.68 {\pm} 0.25^{ab}$	
Glucobrassicanapin	0.27 ± 0.01^{a}	0.26 ± 0.01^{a}	0.24 ± 0.00^{b}	
Glucoerucin	0.70 ± 0.11^{ab}	0.60 ± 0.02^{b}	0.80 ± 0.02^{a}	
Glucohirsutin	$0.25 {\pm} 0.13^{a}$	0.11 ± 0.02^{a}	0.17 ± 0.02^{a}	
Glucoraphasatin	0.02 ± 0.02^{b}	$0.12 {\pm} 0.01^{a}$	0.03 ± 0.00^{b}	
Glucobrassicin	6.87 ± 0.09^{a}	4.96 ± 0.15^{b}	6.92 ± 0.19^{a}	
4-Methoxyglucobrassicin	$5.62 {\pm} 0.11^{a}$	3.37 ± 0.19^{b}	$5.51 {\pm} 0.28^{a}$	
Glucoberteroin	0.50 ± 0.02^{b}	0.39±0.01°	0.53 ± 0.00^{a}	
Gluconasturtiin	$0.50\!\pm\!0.03^{ab}$	0.53 ± 0.05^{a}	0.44 ± 0.00^{b}	
Neoglucobrassicin	2.30±0.01°	2.74 ± 0.13^{b}	3.20 ± 0.00^{a}	
Total	41.59±0.41ª	37.24±0.21 ^b	37.21±0.67 ^b	

Duncan's multiple range test reveals that using different letters signifies a notable distinction in means (p < 0.05).

Table 2: The concentration of carotenoids (μ g/g dry wt.) in kale sprouts irradiated with white, blue, and red LED light 10 DAS

Carotenoids	Concentration (µg/g DW)				
	White-LED	Blue-LED	Red-LED		
Violaxanthin	59.38±2.45 ^b	41.43±3.75°	71.20±1.52ª		
Lutein	1034.72±83.26ª	947.49 ± 48.97^{a}	1052.06 ± 2.46^{a}		
Zeaxanthin	16.05±1.18°	22.06±1.19 ^b	53.70 ± 0.92^{a}		
13-cis-	148.08 ± 9.08^{a}	114.60 ± 4.46^{b}	113.50±3.63 ^b		
β-carotene					
α -carotene	31.05 ± 1.66^{a}	28.45 ± 1.44^{ab}	$25.69 \pm 0.40^{\circ}$		
β-carotene	1935.13±87.21ª	1809.35±58.44 ^b	1678.86±26.10°		
9-cis-	116.86 ± 9.79^{a}	104.24 ± 5.81^{ab}	96.45±0.68 ^b		
β-carotene					
Total	3341.27±206.96ª	3067.64±116.37 ^b	3091.44±27.87 ^b		

Duncan's multiple range test reveals that using different letters signifies a notable distinction in means (p < 0.05).

length and fresh weight were achieved in sprouts irradiated with red LED light. However, these findings do not agree with Li *et al.* (2012), Park *et al.* (2020b), and Tan *et al.* (2020), who found that blue LED light benefits vegetation and promotes Table 3: The concentration of phenolic acids (μ g/g dry wt.) in kale sprouts irradiated with white, blue, and red LED light 10 DAS

Carotenoids	Concentration (µg/g DW)			
	White-LED	Blue-LED	Red-LED	
Chlorogenic acid	45.78±0.73 ^a	39.63±0.56°	42.01±0.81 ^b	
Ferulic acid	4.84 ± 0.05^{a}	5.35 ± 0.43^{a}	4.80 ± 0.11^{a}	
Benzoic acid	7.48 ± 0.62^{a}	$2.52 {\pm} 0.18^{b}$	1.92 ± 0.09^{b}	
Total	52.10 ± 5.71^{a}	44.98 ± 0.78^{b}	46.81±0.83 ^b	

Duncan's multiple range test reveals that using different letters signifies a notable distinction in means (p < 0.05).

elongation growth in brassicas plants, whereas red LED light supports reproductive growth.

LED lights influence GSL content. However, the response varies depending on the type of glucosinolate (Demir et al., 2023). Similar results were found in the present study, where the content of GSLs varied in different LED light exposures. Furthermore, Cartea and Velasco (2008) found that the structure and amount of glucosinolates varied significantly between B. oleracea sprouts. We found a high content of aliphatic GSLs (progoitrin), and sprouts submitted to a white LED light irradiation presented the high total content of GSLs in the current study. These results do not agree with those of Cartea and Velasco (2008), who found that different B. oleracea varieties contain glucobrassicin and glucoiberin, which essentially contain significant quantities of sinigrin. Additionally, Lee et al. (2016) found that kale, under blue and red LED light irradiation, produced considerably high levels of GSLs. However, according to Park et al. (2019), sprouts of B. napus grown under white, blue, and red LEDs contained similar levels of total GSLs. Qian et al. (2016) discovered that the roots of Chinese kale sprouts experienced an increase in the beneficial glucoraphanin content when exposed to blue LED light. Additionally, this light source had the advantage of reducing the unwanted gluconapin in the shoots. These effects were not observed with dark, white, or red lights. These results are similar to those we found in the present study. According to Sathasivam et al. (2023), the individual GSLs content, such as 4-hydroxyglucobrassicin, glucoerucin, 4-methoxyglucobrassicin, and neoglucobrassicin, were improved in kohlrabi sprouts exposed to blue LED light compared to those exposed to white and red LED light. These results are far different from those found in the current study. The findings indicate that most *Brassicas* have similar GSL compounds. Nevertheless, varying LED lights can influence individual and total GSL content accumulation.

Carotenoids are plant pigments categorised as secondary plant compounds that play a crucial role in enhancing human well-being and are thus significant for the overall quality of vegetables (Cazzonelli, 2011; Fiedor & Burda, 2014). In the present study, we found β -carotene as a carotenoid observed in high levels, and it belonged to kale sprouts exposed to white LED light irradiation, followed by rutin in red LED light. Similar results were found by Sathasivam et al. (2023), who observed that the highest amount of total carotenoid was in kohlrabi sprouts irradiated with white LED light, and the individual carotenoids found with the highest contents were β -carotene and lutein. Likewise, Frede *et al.* (2018) observed that higher carotenoid quantities were measured under white LED compared to blue or red LED light irradiation. Another study found that lettuce and Komatsuna had higher total carotenoid levels when exposed to white and red lights. In contrast, when exposed to blue light, spinach had the highest carotenoid levels (Ohashi-Kaneko et al., 2007). However, Li et al. (2012) discovered contrasting outcomes, as they observed that exposing Chinese cabbage microgreens to blue LED light resulted in an augmentation in carotenoid production. Additionally, in lettuce and broccoli sprouts, blue light increased the carotenoid content (Johkan et al., 2010; Kopsell & Sams, 2013). Furthermore, (Frede et al., 2023) found that blue light increases the transcription of carotenoid biosynthesis genes in five Brassica spouts. Based on current and past findings, choosing the proper LED lighting to enhance plants' overall and individual carotenoid levels may vary depending on the species.

The content of PAs in Brassica microgreens grown under different LED light quality conditions varies widely. We detected and quantified three PAs, including chlorogenic acid, in high quantities in kale sprouts under white LED light irradiation. Similar results were found with Sathasivam et al. (2023), who found high levels of chlorogenic acid in kohlrabi sprouts in white LED light conditions compared to red and blue LED light. However, the total PAs were found in sprouts irradiated with blue LED light treatment. Different results from Olsen et al. (2009) and Lin and Harnly (2010) the most common PAs in Brassica vegetables are p-coumaric, sinapic and ferulic acids. Additionally, Kim et al. (2015) Discovered that in *B. rapa* subsp. *pekinensis*, *p*-hydroxybenzoic acid was the most abundant PA measured under blue LED light, followed by chlorogenic acid under white LED light. Furthermore, Yeo et al. (2018) stated that cowpea sprouts grown under white LED light presented the lowest level of PAs. In another study, Cuong et al. (2019) showed that most of the PAs found were quercetin and gallic acid in wheat sprouts under white LED light irradiation. According to Park et al. (2020b), A. rugosa plantlets exposed to white LED lights accumulated the highest amounts of rosmarinic acid and tilianin. Additionally, Park *et al.* (2020a) observed that in *B. juncea* sprouts, exposure to a blue LED light enhanced the production of most PAs. Researchers can potentially improve the concentration of a particular compound by cultivating kale sprouts under appropriate LED lighting. These findings offer potential avenues for improving the quality of these compounds in kale sprouts.

CONCLUSION

Artificial light can be used as a replacement for natural light in plants. Different light sources have varying effects on plant morphology and the accumulation of certain compounds. White LED light positively affected the concentration of GSLs, carotenoid and PAs in kale sprouts, while red LED light positively affected morphology. This study provides a strategy for improving phytochemicals in kale. Future studies can explore other types or combinations of LED light for further insights. Additionally, there is a need for more research on the effects of LED illumination on other plant compounds such as chlorophyll, anthocyanin, protein, vitamin, mineral and antioxidant activity.

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