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Research article

Physicochemical properties and bioactive compounds of purple rice (*Oryza sativa* L.) seedlings grown under light-emitting diodes

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Abstract

Importance of the work: There has been increasing interest in using light-emitting diodes (LEDs) to aid in the development of bioactive compounds in a variety of plants to enhance crop nutritional quality.

Objectives: To investigate the effects of LEDs on the physicochemical properties and bioactive compounds of rice seedlings.

Materials & Methods: Purple rice seeds of *Oryza sativa* L. were grown under LEDs at different wavelengths (white, red, blue, and red+blue LEDs) for 12 hr/d, while the other 12 hr were in darkness. The physical properties, chemical properties and free-radical scavenging activity of the purple rice seedlings were investigated.

Results: Applying exposure to LEDs during the seedling stage significantly ($p < 0.05$) affected the growth and physicochemical quality of the rice seedlings. Seedlings grown under the red LEDs developed an increased plant height and reducing sugar accumulation. The leaf width, total soluble solids content, chlorophyll content (22.70 mg/g), total phenolic content (5.73 mg gallic acid equivalents/g), and free-radical scavenging activity (79.41%) were significantly higher in rice seedlings grown under the blue LEDs than those under the other LED conditions. The red, blue and red+blue LEDs had no significant effects on the hue angle, ascorbic acid content, titratable acidity or pH.

Main finding: Blue LEDs can be considered as an abiotic elicitor for growing purple rice seedlings, with the advantage of an increase in the secondary metabolites.

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Introduction

Rice (*Oryza sativa* L.) is an important cereal crop food source for populations all over the world (Jehangir et al., 2022). In Thailand, colored rice (known as pigmented rice) is applied for therapeutic purposes, especially in the northern and northeastern areas of the country (Laokuldilok and Kanha, 2015; Melini and Acquistucci, 2017). Pigmented rice is identified by the color of the rice's pericarp, which varies from red to dark purple, with this rice containing high levels of bioactive compounds such as anthocyanins and phenolic compounds; consequently, pigmented rice has become popular in recent years (Mbanjo et al., 2020). These compounds have been recognized as health-enhancing substances due to their antioxidant, anti-inflammatory, anticancer and hypoglycaemic effects (Piluzza and Bullitta, 2011).

Nowadays, consumers are becoming increasingly health-conscious, with many healthy products from plant parts with high bioactive compounds and antioxidant activity being launched onto the market and of these, wheatgrass juice at the jointing stage has been consumed as a functional food since 1980 (Falcioni et al., 2002). Juice squeezed from wheatgrass and wheat-sprout extracts contains higher phytonutrients and antioxidant activity than seeds after sprout detachment or non-sprouted seeds (Falcioni et al., 2002; Sytar et al., 2018). The sprouts of the purple wheat genotypes had the highest specific anthocyanidins compared with the blue and yellow versions (Sytar et al., 2018). Wheat production is marginal in Thailand, due to the unfavorable climatic conditions (United States Department of Agriculture, 2021). Therefore, purple rice with high antioxidant activity may be a useful alternative to wheat grains.

Light is known as an environmental factor that triggers plant development and morphogenesis. The spectrum composition or wavelengths of light strongly influence plant development (Chen et al., 2016). Light-emitting diodes (LEDs) have often been applied as a light source emitting near infrared (NIR) radiation for indoor plant growth (Landi et al., 2020). Chlorophylls are the primary photosynthetic pigments in plants and play a key role in light-energy absorption; it has been reported that the chlorophylls biosynthesis pathway is induced by light, and different spectra have a great influence on the formation of photosynthetic pigments (Chen et al., 2016). Blue and red LEDs have been widely selected, as both wavelengths are efficiently absorbed by chlorophylls (Landi et al., 2020). In rice seedling leaves, the concentration of chlorophyll is greater under blue and red+blue LEDs lighting treatments (Chen et al., 2016).

Monochromatic light affects not only a plant's photosynthetic performance; it also modulates the biosynthesis of the photoprotective compounds (Landi et al., 2020). Recently, LED irradiation has been used to induce the accumulation of bioactive compounds in several herbs, fruits and vegetables. Red-light irradiation positively influenced metabolic processes and contributed to a higher content of health-promoting compounds in tomatoes (Panjai et al., 2019). Blue LED light increased the amount of essential phytochemical components in broccoli microgreens (Kopsell and Sams, 2013). In addition to monochromatic light, red LEDs in combination with blue LEDs maintain a higher antioxidant level and phenylalanine ammonia-lyase enzyme activities in lemon balm, compared to those irradiated under greenhouse light (Ahmadi et al., 2020).

Based on its antioxidant property, colored rice grass juice in some cultivars grown under natural light has been reported to exhibit greater antioxidant activity than grass juice obtained from white rice and wheat (Khanthapok et al., 2015). Chuchird et al. (2017) reported the active compounds of six kinds of Thai black glutinous rice grains (Kum King, Kum Doisaket, Kum Phayao, Kaoneawdam Homphukeaw, Kaokumpae and Kaoneadam Leumpua) under natural light. Among these cultivars, Kum Phayao had the highest total phenolic compounds and a high antioxidant activity. However, the chlorophyll contents of grass drinks from three varieties of Thai rice grown under natural light were very low compared to wheatgrass juice (Wangcharoen and Phimphilai, 2016). Based on the current literature, there has been no reports on the impact of LEDs on the induction of bioactive compounds and the physicochemical qualities of purple rice seedlings. Good knowledge of some of the physicochemical properties and the phytochemical content of purple rice seedlings should be useful for further application in functional food products. Therefore, the current study aimed to investigate the effects of LED light at different wavelengths on the physicochemical properties and bioactive compounds of purple rice seedlings. In addition, the proximate composition and energy of purple rice seedlings were evaluated for their feasible application in healthy food products.

Materials and Methods

Plant materials and treatment

Seeds of purple rice (*Oryza sativa*) cv. Kum Phayao were obtained from a commercial rice mill in Chun district, Phayao province, Thailand. Rice seeds with a germination rate higher than 80% were washed and soaked overnight in tap water. The seeds were germinated and planted in a vermiculite medium in plastic trays (20 cm × 30 cm × 6 cm) and supplied with tap water under different LED lights: white LEDs (control), red LEDs (650–660 nm), blue LEDs (450–460 nm) and a combination of red+blue LEDs (red:blue, 70%:30%) at ambient temperatures (day/night conditions were 30°C/ 25°C, 70–80% relative humidity) with a 12 hr photoperiod for 2 wk. Lighting-array LEDs (Growlab Agritech Co., Ltd.; Samut Prakan, Thailand) were adjusted to 30 cm above the plants, under a photosynthetic photon flux density (PPFD) held at on approximately 55 µmol/m^2/s measured using an LI-COR Quantum meter (Li-Cor Inc.; Lincoln, NE, USA). Approximately 100 rice seedlings were grown in each treatment and the experiment was performed three times.

Purple rice seedlings were harvested after 14 d, when the maximum %extraction yield could be expected, according to Tanprasit et al. (2019). Samples were cut 1.5 cm above the vermiculite and cleaned in deionized water. Juice extracts were prepared by mixing fresh rice grass and deionized water (1:2) to determine the pH, titratable acidity, total soluble solid, reducing sugar and ascorbic acid. Then, the mixture was homogenized at maximum speed and filtered through three layers of white cloth.

Physical property measurements

The plant height and leaf width of 20 rice grass plants were measured during growth using a vernier caliper. Plant height was measured from the ground to the top of the leaf. The widest part of the leaves was used to measure their width.

Color determination

The color of the rice seedlings was recorded as $L*$ (brightness), $a*$ (red-green), and $b*$ (yellow-blue) using a ColorQuest XE (Hunter Associates Laboratory Inc.; Reston, VA -$b*$ USA). The hue angle was calculated as 180º + tan^{-1} ($b*/a*$) when $a* < 0$ and $b* > 0$.

Total soluble solid and titratable acidity

The total soluble solids (TSS) of the rice seedling juice was determined using a digital refractometer (PAL-1, ATAGO; Tokyo, Japan) and was recorded as °Brix. The titratable acidity (TA) of the rice seedling juice was determined based on titration with 0.1 N NaOH and a phenolphthalein indicator.

Reducing sugar

The reducing sugar of the rice seedling juice was determined using the dinitrosalicylic (DNS) colorimetric method. A sample (2 mL) of juice was mixed with a DNS reagent. The mixture was incubated in a boiling water bath for 15 min. The absorbance at 550 nm was measured using an ultraviolet-visible spectrum (UV-Vis) spectrophotometer (Evolution 201; Thermo Scientific; Madison, WI, USA). The concentration was evaluated using a glucose standard curve.

Chlorophyll content

The chlorophyll content of the rice seedlings was determined according to Arnon (1949), with some modifications. A sample (1 g) of fresh grass was cut into small pieces and ground using a mortar and pestle in 0.85% acetone. Then, the homogenate sample was centrifuged at 10,000×*g* for 10 min. The supernatant was measured using an Evolution 201 UV-Vis spectrophotometer (Thermo Scientific; Madison, WI, USA) at 663 nm and 645 nm. The total chlorophyll content was calculated using Equation 1 and expressed as milligrams per gram fresh weight (FW):

$$\text{Total chlorophyll} = (20.2A_{645} + 8.02A_{663}) \times \text{dilution factor} / 1{,}000 \quad (1)$$

where A_{645} and A_{663} are the absorbance at 645 nm and 663 nm, respectively.

Ascorbic acid content

The ascorbic acid content was assayed using the indophenol method (Nielsen, 2017). A sample (2 mL) of rice-seedling juice was mixed with 5 mL of metaphosphoric acid-acetic acid solution and titrated with 2,6-dichlorophenolindophenol (dye solution) until a light but distinct rose-pink color persisted for more than 5 s. The ascorbic acid content of the sample was calculated using Equation 2 and expressed in micrograms per milliliter:

$$\text{Ascorbic acid content} = (X - B) \times (F / E) \times (V / Y) \times 1{,}000 \quad (2)$$

where X is the volume of the sample titration; B is the volume of the blank titration; F is the solution titer of dye use to titrate ascorbic acid; E is the sample volume; V is the volume of the initial assay solution; and Y is the volume of the sample aliquot titrated.

Total phenolic content determination

The total phenolic content was analyzed according to the method of Ketsa and Atantee (1998). A sample (1 g) of fresh rice seedling was homogenized in 80% ethanol and centrifuged at 12,000×*g* for 20 min at 4°C. The supernatant was mixed with Folin-Ciocalteu phenol solution for 3 min. After this, 2 mL of 10% (weight per volume) sodium carbonate was added to the mixture and incubated in the dark at room temperature for 60 min. The total phenolic content (TPC) was determined spectrophotometrically at 760 nm and evaluated using a standard curve-provided gallic acid. The contents were expressed as milligrams of gallic acid equivalents per gram fresh weight of plant material.

Determination of antioxidant activity

The antioxidant activity was determined using the DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay according to the method of Brand-Williams et al. (1995), with slight modification. The supernatant was obtained using the same method as for the total phenolic content measurement. The supernatant (150 µL) was added to 2,850 µL DPPH in methanol. The reaction mixture was mixed and allowed to stand in the dark overnight at room temperature. The absorbance was measured at 515 nm using the same UV-Vis spectrophotometer as above. The percentage of inhibition was calculated against a blank and assessed as antioxidant activity using Equation 3:

$$\text{DPPH inhibition (\%)} = [(\text{A blank} - \text{A sample})/\text{A blank}] \times 100 \quad (3)$$

where A blank is the absorbance of the control reaction (containing all reagents except the test sample) and A sample is the absorbance of the reaction mixture.

Proximate composition

Proximate analysis of fresh rice seedlings was used to determine the moisture, crude protein, crude fat, ash, and crude fiber contents, according to the methods of Association of Official Analytical Chemists (2016). The total carbohydrate of the fresh rice seedlings was obtained by subtracting the value of the moisture, fat, protein, ash, and crude fiber contents from 100 (Association of Official Analytical Chemists, 2016). The energy in kilocalories per 100 g was calculated based on Equation 4 (Tanprasit et al., 2019).

$$\text{Energy} = (\%\text{protein} \times 4) + (\%\text{fat} \times 9) + (\%\text{carbohydrate} \times 4) \quad (4)$$

Statistical analysis

An analysis of variance to evaluate the treatment effects was performed followed by Duncan's multiple-range test in the SPSS software program (version 18; SPSS Inc.; Chicago, Il, USA). The tests were considered significant at $p < 0.05$.

Results and Discussion

Effects of light-emitting diode illumination on physical properties of purple rice seedling

The plant height of rice seedlings grown under different LEDs is shown in Fig. 1A. Red light significantly increased the plant height throughout the 2 wk period, while treatment with blue and red+blue light produced a similar plant height as the red+blue and white LEDs. As shown in Fig. 1B,

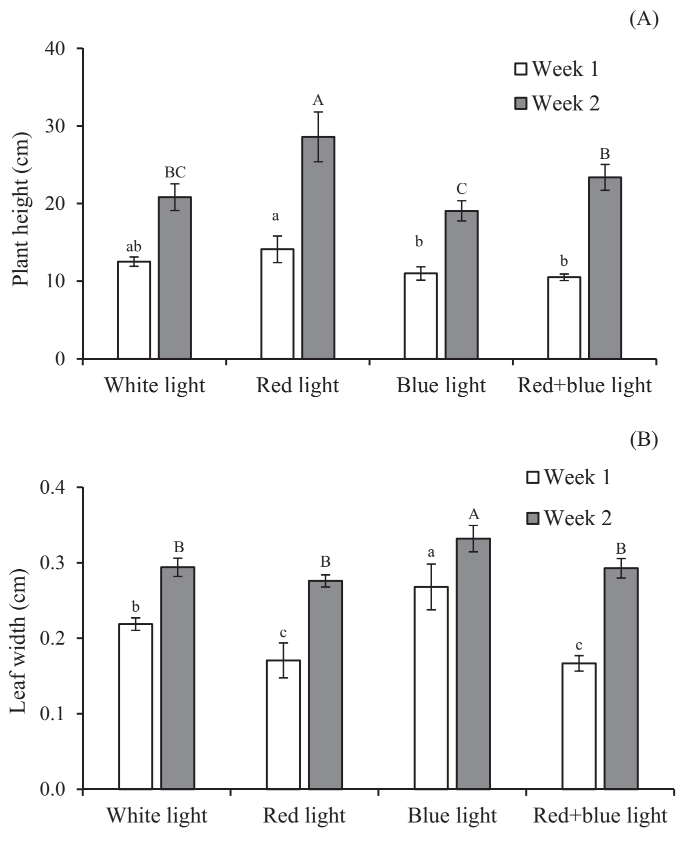

Fig. 1 Rice seedlings grown under different light-emitting diode lights for 2 wk: (A) plant height; (B) leaf width, where error bars show mean±SD ($n = 5$). Different lowercase or uppercase letters above bars indicate significant ($p < 0.05$) differences among treatments within each growing period

the leaf width of the rice seedling was affected by different LEDs. Blue light treatment significantly induced leaf width during the first and second weeks. Similarly, Li et al. (2012) determined that blue LEDs benefited vegetative growth, while red LEDs and red+blue LEDs supported reproductive growth in non-heading Chinese cabbage. Fan et al. (2013) reported that with cabbage, red light promoted stem elongation, while blue light inhibited it because blue light plays an important role in both photomorphogenesis and dry matter production, due to the promotion of stomatal opening (Matsuda et al., 2004; Kopsell and Sams, 2013; Landi et al., 2020).

Ahmadi et al. (2020) showed that combined red and blue lights coincided completely with chlorophyll *a* and *b* and the phytochrome absorption range. Therefore, the improvement of photosystem II efficiency led to an increase in the plant growth and development. This was in contrast to the current results, where the combination of the red+blue LEDs did not show a distinctive plant height and leaf width compared to using the red or blue LEDs alone.

The pericarp color of the Kum Phayao rice used in the current study was dark purple, while the grass was green. The colors of the rice seedlings determined after 2 wk based on values for lightness (L^*), greenness ($-a^*$), yellowness (b^*) and hue angle are shown in Table 1. In addition to $-a^*$, a hue angle at 180° also suggests the green color of the sample. All samples were green in color, as indicated by the hue angle (178.78–178.88°), but there were differences in the shades of green. Rice seedlings being grown under the white LEDs had the significantly highest lightness and yellowness, implying that rice seedlings grown under the white LEDs had a lighter green color than those grown under the red, blue alone or red+blue LEDs light. The green color of the fresh rice seedlings was dependent on the chlorophyll pigmentation.

Effects of light-emitting diode illumination on chemical properties of purple rice seedlings

The TSS content of rice seedlings was greatest under the blue LEDs (Table 2). Supplementary artificial blue light increased the photosynthesis and resulted in a higher carbohydrate content in plants (Li et al., 2015). The current results were consistent with Li et al. (2012) regarding non-heading Chinese cabbage; they reported that the blue LEDs benefited the accumulation of nutritional substances, which may be correlated with the specific plant species.

Rice seedlings grown under the red LEDs had the highest levels of reducing sugar, being twice as high as those grown under other LEDs (Table 2). This might have been because monochromatic red light stimulates the accumulation of photosynthetic products, while inhibiting their translocation out of leaves, thereby elevating the carbohydrate accumulation in leaves (Li et al., 2012; Landi et al., 2020).

LED illumination had no significant effect on the pH (6.2–6.5) and TA (0.11–0.14%) of the purple rice seedlings (Table 2), implying the weak acidic condition of the samples.

Table 1 Color ($L^*a^*b^*$, hue angle) of purple rice seedling grown under different light-emitting diodes (LEDs) for 2 wk

LED	L^*	a^*	b^*	Hue (°)
White	60.28±2.37[a]	-9.12±0.20[a]	24.87±1.13[a]	178.78±0.02[a]
Red	54.81±2.91[b]	-8.62±0.47[a]	21.24±1.71[ab]	178.82±0.05[a]
Blue	52.09±1.34[b]	-9.30±0.36[a]	19.27±1.54[b]	178.88±0.03[a]
Red+blue	55.36±2.85[b]	-8.33±0.72[a]	19.60±3.17[b]	178.84±0.07[a]

Mean±SD ($n = 5$) in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

Table 2 Physicochemical properties of purple rice seedling grown under different light-emitting diodes for 2 wk

LED	TSS (°Brix)	Reducing sugar (µg of glucose/g FW)	Titratable acidity (%)	pH
White	1.00±0.0[b]	1.35±0.23[b]	0.14±0.05[a]	6.19±0.08[a]
Red	1.33±0.6[b]	3.67±0.71[a]	0.13±0.03[a]	6.36±0.19[a]
Blue	2.00±0.0[a]	1.53±0.25[b]	0.11±0.03[a]	6.38±0.14[a]
Red+blue	1.00±0.0[b]	1.24±0.08[b]	0.11±0.03[a]	6.47±0.03[a]

TSS = total soluble solids; FW = fresh weight

Mean±SD ($n = 3$) in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

Effects of light-emitting diode illumination on bioactive compounds and antioxidant activity of purple rice seedlings

The biosynthesis of plant pigments is generally referenced to their specific wavelength absorption patterns. The chlorophyll contents of rice seedlings grown under the red, blue and red+blue LED light sources were higher than those of seedlings exposed to the white LEDs (Table 3). Rice seedlings grown under the blue LEDs had the highest chlorophyll content, followed by those grown under the red, red+blue and white LEDs, respectively. This was attributable to the fact that blue light induced the synthesis of chlorophyll, while red light inhibited chlorophyll synthesis and mediated the chlorophyll degradation pathway in the rice seedling leaves. Landi et al. (2020) revealed that the chlorophyll synthesis inhibition under red LEDs can be avoided by supplementing with blue light. Nevertheless, the current results showed that purple rice seedlings grown under red+blue LEDs could contribute to a higher chlorophyll content than those grown under white LEDs.

Ascorbic acid is generally known as an antioxidant substance found in plants (Akram et al., 2017). In the current study, there were no significant differences in the ascorbic acid contents among the rice seedlings grown under the different LED lights. The ascorbic acid content was in the range 16.25–22.75 μg/mL (Table 3).

The blue LEDs induced significant increases in the TPC and DPPH inhibition activity in the rice seedlings, followed by the red+blue, red and white LEDs, respectively. The DPPH inhibition activity was increased according to the increased TPC (Table 3). The antioxidant potential of the phenolic compounds depends on their redox properties, which allow them to act as hydrogen donors, reducing agents and singlet oxygen quenchers (Piluzza and Bullitta, 2011). Piluzza and Bullitta (2011) found a highly positive correlation between the total phenolic compounds and the antioxidant activity (based on DPPH assay) in 24 medicinal plants. Furthermore, the phenolic content could be used as an indicator of the antioxidant properties of the examined plant species.

In general, the bioactive compounds of plants are synthesized as a secondary metabolite after photosynthesis (Yang et al., 2018). Polyphenols are one plant secondary metabolite that play an important role in resistance against free radicals (Šamec et al., 2021). LED light can also be a stress factor which has a positive effect in increasing the number of important secondary metabolites. For example, studies on other plants showed that a blue wavelength supplement led to an increase in the phenolic compounds and the antioxidant potential in red leaf lettuce (Stutte et al., 2009), red leaf lettuce seedlings (Johkan et al., 2010) and green vegetables (Olle and Virsile, 2013). The current study confirmed other findings that the high antioxidant activity of rice seedlings grown under blue LEDs corresponded with the enhancement of the chlorophyll and the total phenolic content (Table 3). The increase in the total phenolic compounds in blue LED-exposed rice seedlings may have been due to the stimulation of the genes belonging to the phenylpropanoid pathway, which is related to the biosynthesis of phenolic acids (Landi et al., 2020). Another explanation for the higher antioxidant activity of plants exposed to blue LEDs is that the higher energy levels of short wavelengths (for example, blue at 450–460 nm) stimulate oxidative stress, thereby enhancing the scavenging of the reactive oxygen species (Ahmadi et al., 2020).

Water-extract-chlorophyll derivatives have been found to reduce DNA damage by acting as free-radical scavengers and chelating agents to protect human lymphocyte DNA from oxidative damage (Hsu et al., 2005). Therefore, production of phenolic compounds accompanied by total chlorophyll became apparent when the plants were treated with the blue LEDs; the antioxidant capacity of such rice seedlings was higher than for those under the red and red plus blue LEDs.

Table 3 Chlorophyll, ascorbic acid, total phenolic contents and DPPH inhibition of purple rice seedling grown under different light-emitting diodes (LEDs) for 2 wk

LED	Chlorophyll content (mg/g FW)	Ascorbic acid content (μg/mL)	Total phenolic content (mg GAE/g FW)	DPPH inhibition (%)
White	12.01 ± 0.76^{c}	19.50 ± 1.95^{a}	4.14 ± 0.47^{b}	54.25 ± 3.39^{b}
Red	17.43 ± 1.20^{b}	16.25 ± 3.42^{a}	4.55 ± 0.67^{b}	55.20 ± 4.03^{b}
Blue	22.70 ± 1.82^{a}	22.75 ± 5.63^{a}	5.73 ± 0.36^{a}	79.41 ± 3.65^{a}
Red+blue	16.53 ± 2.46^{b}	17.55 ± 3.38^{a}	4.82 ± 0.13^{b}	49.40 ± 3.45^{b}

FW = fresh weight; GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-2-picrylhydrazyl

Mean±SD ($n = 3$) in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different

In the current study, the purple rice seedlings grown under the blue LEDs had a dark green color due to their high chlorophyll content (Tables 1 and 3), in addition to a considerable increase in the TPC and DPPH inhibition activity (Table 3). Therefore, purple rice seedlings grown under blue LEDs light could be considered as functional raw material with antioxidant potential because of their total phenolic and chlorophyll contents and other natural antioxidants.

The proximate analysis of the purple rice seedlings grown under the blue LEDs is presented in Table 4. Those rice seedlings contained a high moisture content, followed by crude fiber, carbohydrates, protein and ash contents, respectively. Compared to a study on white and black glutinous rice sprouts grown under natural light (Tanprasit et al., 2019), the current results showed that the proximate compositions of purple seedings grown under the blue LEDs light were lower in carbohydrates, fat and energy, but higher in protein. Chen et al. (2014) also showed that indica rice seedlings (purple leaf) cultured under blue LEDs had a higher total protein content, compared to those exposed to red+blue LEDs.

Table 4 Proximate composition and energy (mean±SD, n = 3) of purple rice seedling grown under blue light-emitting diodes for 2 wk

Parameter	Amount
Moisture (g/100 g fresh weight)	79.10±0.92
Protein (g/100 g fresh weight)	6.21±0.32
Fat (g/100 g fresh weight)	0.05±0.00
Ash (g/100 g fresh weight)	5.38±0.42
Fiber (g/100 g fresh weight)	28.17±2.84
Carbohydrate (g/100 g fresh weight)	9.26±1.27
Energy (kcal/100 g)	62.34±5.29

In conclusion, the results of this study showed that LED light exposure during the seedling stage significantly affected the growth and physicochemical properties of the purple rice seedlings, compared to the control. Seedlings grown under the red LED light achieved a greater plant height and reducing sugar accumulation, while those grown under the blue LED light achieved a greater leaf width and contents of chlorophyll, phenolic compounds and free-radical scavenging activity. The current findings suggested that value-added products based on purple rice seedlings can be achieved by supplementing their growth with blue LED light. The bioactive compounds produced at the seedling stage of purple rice could be recommended as food supplements for health-conscious consumers. In addition, LEDs can provide a controllable and alternative source of selected single or mixed wavelength photon sources to produce high-value bioactive compounds in greenhouse conditions.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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