



RESEARCH ARTICLE

Effects of substrates and light-emitting diode illumination on the phytochemical content of roselle (*Hibiscus sabdariffa* L.) microgreens

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Abstract

A study was conducted to investigate the effect of substrates and illumination using light-emitting diodes on the total phenolic content (TPC) and total flavonoid content (TFC) of roselle microgreens. Light-emitting diodes such as red, blue, a combination of red and blue (RB) and white were used as artificial lights of the roselle microgreens. Cocopeat, carbonised rice hull (CRH) and vermiculite are the substrates of microgreens. Newly sown seeds were exposed to 48 hr of darkness followed by light exposure. Light exposure of 10 hr was done, depending on the LEDs (red (R), blue (B), RB and white), while 14 hr of darkness until 7 days after sowing (DAS), the time of harvesting (with true leaves). Roselle microgreens were grown under ambient conditions (temperature: 28 ± 3 °C; relative humidity: 60 ± 5 %). For a fresh yield of microgreens, blue LED + CRH and red LED + vermiculite obtained the highest. Roselle microgreens grow best in RB LED + cocopeat to obtain higher TPC and TFC. The antioxidant activity was higher in roselle microgreens grown under cocopeat and vermiculite with either blue, red, or RB LEDs. The availability of the substrate may be considered when combining it with the LED, using the following combinations to obtain high TPC and TFC. For TPC, these include cocopeat + RB, CRH + RB or white and vermiculite + white. For TFC, the suitable substrates are cocopeat and CRH + RB, as well as vermiculite + white.

Keywords: antioxidant activity; LEDs; light quality; phenolic compounds; substrates

Introduction

Interest in microgreen production is increasing due to their nutrient density and health benefits and over time, scientific interest in microgreens has increased (1). Microgreens are small, tender young plants that mature from 7–21 days after germination/sowing. A diverse range of species can be cultivated as microgreens and some are from Brassicaceae, Graminae, Leguminosae, to herbs (2). Microgreens are considered a functional food owing to their varying phytochemicals, including ascorbic acid, carotenoids, phenolics, flavonoids, betalainins, anthocyanins, lutein and β -carotene and contain minerals (2, 3). Roselle can also be a source of microgreens and locally, is commonly produced for its flowers as a source of wine and a souring agent for soups. Locally, roselle petals are commonly used as a souring agent for local soups. In consideration of the benefits, roselle is a promising microgreen.

Growth and phytochemicals, such as total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity, have complex variations due to abiotic factors such as growing media, moisture level and light conditions (1, 4). However, in this study, substrates and light colors, using light-emitting diodes (LEDs), were used. Substrates, however, are essential to provide an anchor for the microgreens and a source of moisture content. Cocopeat,

carbonised rice hull (CRH) and vermiculite were used in the study. Cocopeat contains phosphorus, potassium, calcium, magnesium and sodium (5, 6). It should be noted that the mineral compositions vary, depending on the cocopeat source. The CRH contains phosphorus, potassium, calcium and magnesium (7). In a previous report, vermiculite was reported to contain nitrogen, phosphorus, potassium, magnesium, aluminum and iron (8, 9). These substrates and LEDs are used in horticultural plant production and it can be used for roselle microgreens to maximise the full potential in obtaining growth and phytochemicals.

LEDs are a source of artificial light in the cultivation of microgreens and sprouts. Red and blue LEDs are commonly used in horticulture due to their effect on photosynthesis and photoreceptor activation (10, 11). It was previously reported that there are photoreceptors, such as phytochromes, cryptochromes and phototropins affect the stimulation or inhibition of the growth and metabolism (12). LEDs have a significant impact on the plant receptors and on the synthesis of bioactive compounds, such as phenolic compounds (13). Likewise, LEDs can act as an elicitor that triggers the production of carotenoids and phenolics and it was reported that the combination of red and blue light effectively boosts these compounds in eggplant (14). Previous studies have reported that LED affected the TPC and antioxidant activity (15–17).

It was reported that illumination with white LEDs in kale microgreens produced high amounts of carotenoids, but blue LEDs dominated in the production of phenolic compounds and antioxidant activity (16). However, they further observed that the antioxidant activity in blue LED was similar to white LED. Moreover, blue and white LEDs displayed a higher level of TPC than red LED in mustard sprout (18). Exposure of cabbage microgreens to white LED produced a higher TPC than combined with red or blue LEDs (17). They have reported that the antioxidant activity was higher in cabbage microgreens grown in white or white + red LEDs. The responses of TPC from different LEDs signify a species-specific response. In roselle microgreens, suitable combinations of LED and substrate are needed to maximise their uses and the effect of LEDs on the phytochemicals may have a varying impact on the roselle microgreens. Thus, a study was conducted to evaluate the effect of substrates (cocopeat, CRH and vermiculite) and LEDs on the TPC, TFC and antioxidant activity of roselle microgreens.

Materials and Methods

Source of roselle seeds and planting

The seeds were sourced from a local grower in Ilocos Norte. Seeds were surface sterilised with 1 % sodium hypochlorite for 30 sec and afterward the seeds were washed 3 times using distilled water.

Seeds were sown directly in trays (20 × 14 × 5 cm, L × W × H) with a corresponding substrate. After sowing, the seeds were covered with a thin layer of a similar substrate. Commercialised substrates used were cocopeat, CRH and vermiculite and procurement was done online and in local retail stores in Ilocos Norte.

Growing conditions

Newly sown seeds were exposed to 48 hr of darkness followed by light exposure. Ten hours of light exposure, depending on the LEDs (red (R), blue (B), RB and white), were employed, whereas 14 hr of darkness until 7 days after sowing (DAS), the time of harvesting, wherein microgreens have shown true leaves. Roselle microgreens were grown under ambient conditions (temperature: 28 ± 3 °C; relative humidity: 60 ± 5 %) of the post harvest and seed technology laboratory room.

The light intensity for each LED was red - 98.5, blue - 702.25, red + blue - 778 and white - 714.75 lux. The spectral composition of red and blue LEDs is 430–480 nm, 620–700 nm, respectively.

Microgreen height and leaf length

The height of 20 microgreen plants was measured from the substrate up to the leaf tip and the leaf length was measured from the leaf point attachment to the leaf tip using a ruler.

Chlorophyll content

A chlorophyll meter was used to measure the chlorophyll content of the roselle microgreens and expressed as SPAD index. Measurement was done 7 DAS.

Root length per microgreen

A ruler was used to measure the root length of the microgreen from the point of attachment to the root tip.

Fresh weight per microgreen and fresh yield

A digital weighing scale was used to record the fresh weight per microgreen and fresh yield (kg/m²). Roselle microgreens with true leaves were harvested manually using a sterilised pair of scissors.

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity

The TPC and TFC, as well as the antioxidant activity of the roselle microgreens, were analysed using a previously established method (19). Freeze-dried samples were used to analyse the phytochemicals of roselle microgreens. Gallic acid and quercetin were used as standards for TPC and TFC, respectively. The DPPH assay was used to assess antioxidant activity.

Statistical analysis

The data obtained were analysed using a completely randomised design with 4 replications. The STAR program was used to analyse the data. Treatment means were compared using the least significant difference (LSD) test at $p \leq 0.05$.

Results and Discussion

Microgreen height and leaf length

Microgreen height and chlorophyll content were significantly affected only by LEDs, with no significant interaction effect (Table 1). Blue and red LEDs showed taller microgreens than those illuminated with RB and white LEDs. RB LED exhibited the shortest roselle microgreens among the LEDs. Interestingly, the effects of LEDs on the chlorophyll content were the reciprocal effect as compared to the microgreen height, wherein the RB LED produced the highest and the blue and red LEDs exhibited the lowest. It was reported that combinations of red and blue lights effectively improved the chlorophyll content and photosynthetic efficiency than the red or blue light alone (20). This was also reported by another study, wherein red and blue light combinations displayed

Table 1. Effect of light-emitting diodes and substrates on microgreen height and chlorophyll content of roselle microgreens

LED/Substrate	Microgreen height (cm)	Chlorophyll content (SPAD index)
Light-emitting diodes (a)		
Blue	10.20 a	25.41 c
Red	10.54 a	25.51 c
RB	8.17 c	29.40 a
White	8.75 b	27.63 b
Substrate (b)		
Cocopeat	9.26 a	26.76 a
Carbonised rice hull	9.59 a	26.60 a
Vermiculite	9.38 a	27.61 a
LED × substrate (c)	ns	ns
CVa (%)	4.36	3.77
CVb (%)	4.62	4.60
CVc (%)	7.07	5.64

Means with different letters in a column indicate a significant difference at $p \leq 0.05$ using the least significant difference test.

high chlorophyll contents in tomato (21).

Leaf and root lengths

Longer leaves were observed in RB and white LEDs if grown in CRH and RB LED using vermiculite (Fig. 1A). Whereas roselle microgreens grown under cocopeat exhibited similar leaf length irrespective of the LEDs. However, across substrates, vermiculite + white LED and vermiculite and CRH + RB LED showed longer leaves. Other combinations of substrates and LEDs displayed shorter leaves. Results show that combinations of substrates and LEDs play a significant role in the leaf length. Previous reports have shown that light colors have an impact on plant growth with the contribution of the photoreceptors and genes in the leaf elongation (22–25). It is

recommended that further studies are needed in the investigation of photoreceptors and the genes involved in leaf elongation.

However, across substrates and LEDs produced a similar root length of roselle microgreens (Fig. 1B). It indicates that irrespective of the substrate and LED to be used for roselle microgreens exhibited the same length of roots.

Fresh weight per microgreen and fresh yield

Combined red and blue LEDs resulted in a higher fresh weight of microgreens grown in CRH than the other substrates (Fig. 1C, D), which was also reported in buckwheat microgreens (26). It was reported that the activity of ribulose-1, 5-bisphosphate (Rubisco) plays a significant role in the physiological process, photosynthesis. Rubisco helps to obtain a high biomass. A previous study reported that exposure of tomato seedlings to blue light showed higher amounts of Rubisco with low biomass (27). This indicates that a lower Rubisco amount may produce a higher fresh weight and if this is the basis, it can be observed that fresh weight was high in red and blue LEDs. In connection with the combination of substrate + LED, observations are as follows: CRH + blue and RB LEDs and vermiculite + red and white LEDs. However, a further analysis of the Rubisco activities is recommended using the same setups.

TPC, TFC and antioxidant activity

TPC of roselle microgreens was significantly affected by the LEDs and substrates (Fig. 2A). RB LED exhibited a higher TPC in every substrate, except in vermiculite, wherein the white LED produced the highest TPC among the other LEDs. Combinations of blue LED + CRH, RB LED + cocopeat and white and red LEDs + any of the substrates displayed high amounts of TPC. Growers have the option of using any of the available substrates in combination with these LEDs to produce higher levels of TPC.

In every substrate, RB LED was higher in cocopeat and CRH,

while white LED was highest in vermiculite. The result of the study contradicts a previous study, wherein blue LED exhibited higher TPC levels than white and red LEDs in kale microgreens (16). The TPC in kale microgreens was 2 times higher than that of roselle microgreens in the present study. The significant variation may be due to the crop species and light illumination differences. In another study, blue and white LEDs displayed a higher level of TPC than red LED in mustard sprouts, which showed similarity in the current study, particularly those roselle microgreens grown in vermiculite + white LED (18). Higher TPC was produced by lighting the sprouts with blue (28, 29), but others reported that it was higher in red light (30). The enhancement of TPC indicates that it is a species-specific response.

However, a high TFC was produced by growing roselle microgreens in either cocopeat or CRH, combined with RB LED (Fig. 2B). Whereas white LED and vermiculite showed a higher TFC than when combined with the other LEDs. Combinations of LED and substrate, such as blue and red LEDs + CRH, RB LED + cocopeat and white LED + CRH or vermiculite, obtained the highest TFC. However, it was reported that red and blue lights effectively enhanced the phenolics and flavonoids, which were observed in eggplant and it was observed in the study that RB LEDs had a high TPC and TFC in the study (14). The interactions of LED and substrate may be due to the nutrient composition and availability in the latter that contributed to the TPC and TFC and which may trigger the enhancement with the former. Although there was no significant impact of LEDs and substrates to the root elongation of roselle microgreens.

Roselle microgreens had a high antioxidant activity in the following LED and substrate combinations: blue LED + cocopeat or vermiculite, red LED + vermiculite, RB LED + vermiculite and white LED + CRH or vermiculite (Fig. 2C). However, in every substrate, such as cocopeat, the blue LED had the highest antioxidant activity. In

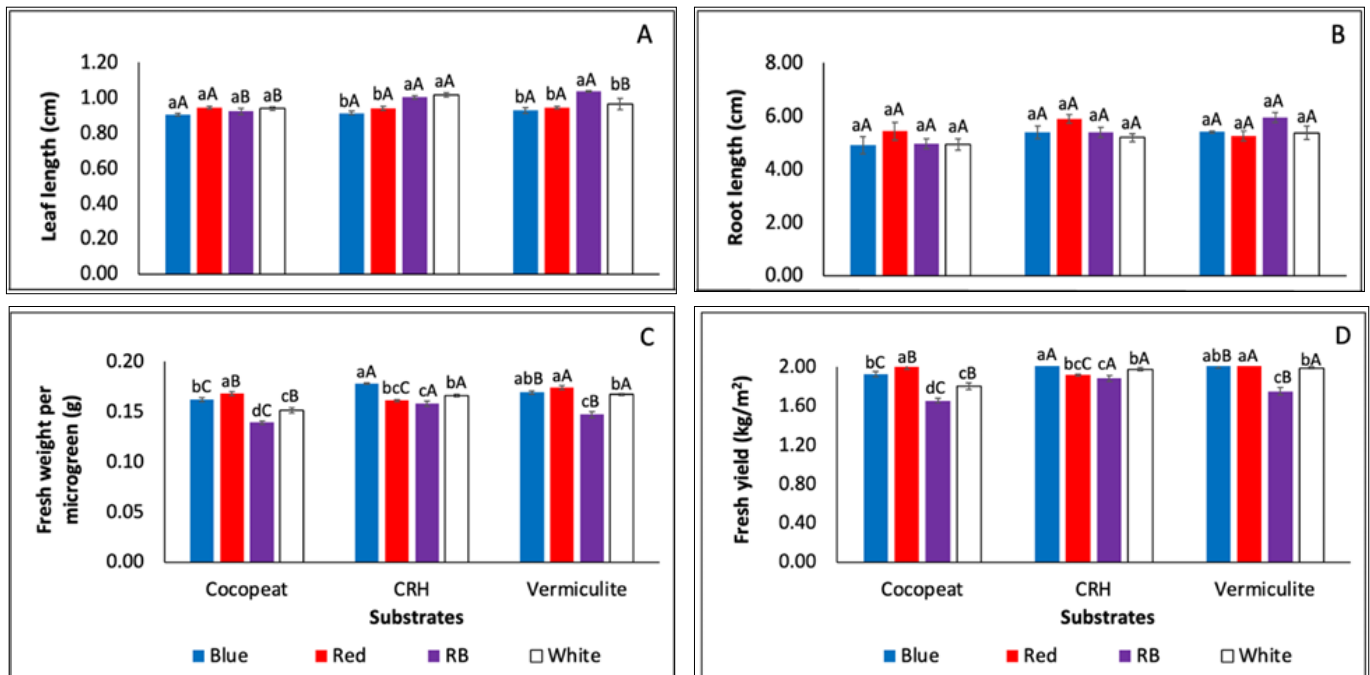


Fig. 1. Selected growth and yield parameters of roselle microgreens.

(A) Variation of leaf length of roselle microgreens as influenced by the light-emitting diodes and substrates; (B) similarities in root length of roselle microgreens irrespective of light-emitting diodes and substrates; (C) differences in fresh weight per microgreen; (D) fresh yield illuminated with light-emitting diodes grown in different substrates.

Bars with different lowercase and uppercase letters indicate significant difference among light-emitting diodes and substrates respectively, using the Least Significant Difference test at $p \leq 0.05$.

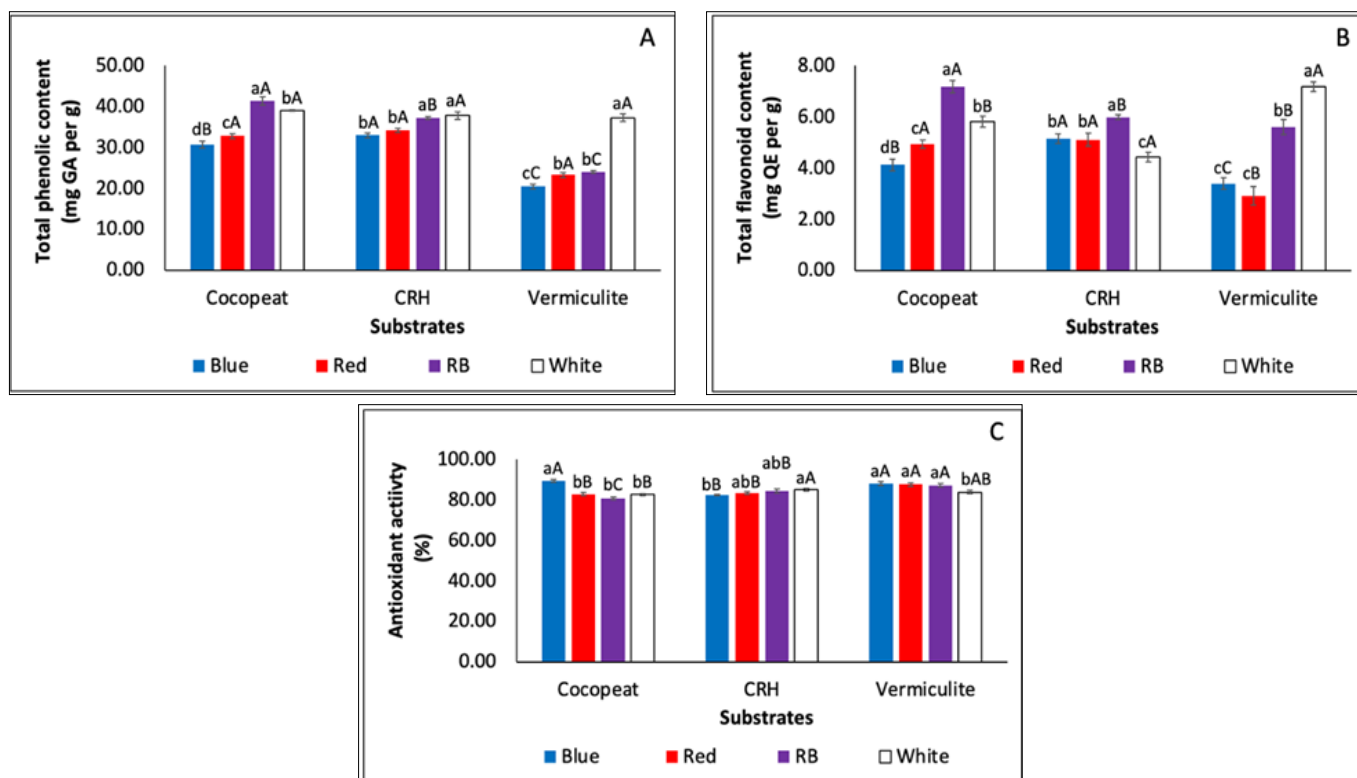


Fig. 2. Variations on the phytochemicals and antioxidant activity in roselle microgreens as affected by substrates and LEDs.

(A) Varying levels of TPC; (B) TFC in roselle microgreens grown under varying substrates and LEDs; (C) varying effects of substrates and LEDs on the antioxidant activity in roselle microgreens.

Bars with different lowercase and uppercase letters indicate significant difference among light-emitting diodes and substrates respectively, using the Least Significant Difference test at $p \leq 0.05$.

CRH, white LED showed a similar antioxidant activity to red and RB LEDs. Growing roselle microgreens in vermiculite using any of the LEDs, except white, produced better antioxidant activity.

It was previously reported that the antioxidant activity was observed in kale microgreens grown under blue LED, which showed a similarity to the study wherein the blue LED had the highest antioxidant activity in roselle microgreens grown under cocopeat (16). But in vermiculite, the blue LED showed a similarity with red and RB LEDs, whereas the white LED had the lowest, which contradicts the previous study. Despite the substrate used in the previous study, it was vermiculite. The variation between the previous and current studies may be attributed to differences in growing conditions (e.g., photoperiod duration and LED intensity) as well as species-specific responses. In a separate study, white and combination with red LEDs exhibited a higher antioxidant activity than white + blue LEDs (17).

Conclusion

Higher phytochemicals (TPC and TFC) were attained by using RB LED + cocopeat. Cocopeat and vermiculite with either blue, red, or RB LEDs exhibited a higher antioxidant activity. But for a fresh yield of microgreens, blue LED + CRH and red LED + vermiculite must be used. The availability of the substrate may be considered when combining it with the LED, using the following combinations to obtain high TPC and TFC. For TPC, these include cocopeat + RB, CRH + RB or white and vermiculite + white. For higher TFC, suitable combinations include cocopeat and CRH with RB LED, as well as vermiculite with white LED.

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Authors' contributions

GADS and RJGR carried out the conceptualisation, methodology, investigation and writing original draft. GADS performed the data curation and editing. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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