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Full Paper

Effects of lighting with LED lights of different colours on phytochemical properties of green leaves from red, yellow and white onion (*Allium cepa* L.) cultivars

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Abstract: Onion (*Allium cepa* L.) is a globally consumed vegetable renowned for its functional and medicinal properties. This study investigated the phytochemical content and antioxidant capacity of fresh onion leaves from red (I), yellow (II) and white (III) onion varieties cultivated under red (RL), blue (BL), green (GL) and white (WL) LED lighting. Post-harvest onion extracts were analysed for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. The highest TPC (0.35 ± 0.02 mg gallic acid equivalent/g fresh weight) and TFC (1.57 ± 0.04 mg quercetin equivalent/g fresh weight) were observed in the RLI group, whereas the lowest values were recorded in the GLIII group (0.16 ± 0.03 mg gallic acid equivalent/g fresh weight) and GLI group (0.58 ± 0.05 mg quercetin equivalent/g fresh weight). Antioxidant capacity assessment via the cupric ion reducing antioxidant capacity assay revealed that the RLI group exhibited the highest reducing power with a trolox equivalent antioxidant capacity of 0.4878, surpassing all other treatments. In conclusion LED light colours significantly influence the phytochemical and antioxidant profiles of onion foliage, with red LED light demonstrating a consistently positive effect on bioactive compound accumulation. These findings suggest the potential application of LED lighting technology in onion cultivation for the enrichment of bioactive compounds.

Keywords: LED light, red onion, yellow onion, white onion, *Allium cepa* L., phytochemical properties, total phenolic content, total flavonoid content, antioxidant activity

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INTRODUCTION

The human body requires antioxidants to counterbalance the effects of free radicals, the primary cause of oxidative stress. These molecules play a critical role in neutralising free radicals and preventing the resulting oxidative cellular damage. Under physiological conditions, endogenous antioxidants can be synthesised in sufficient quantities. However, this synthesis may become inadequate due to various endogenous factors such as aging and metabolic disorders, as well as exogenous factors including environmental pollution, radiation and nutritional deficiencies. In such cases it becomes necessary to obtain antioxidants from external dietary sources to compensate for the deficiency [1]. In this context vegetables and fruits are of considerable importance as natural sources of antioxidants in human nutrition [2]. The principal antioxidant compounds present in these foods include tocopherols, flavonoids, phenolic compounds, alkaloids, chlorophyll, nitrogen-containing compounds, polyfunctional organic acids and carotenoids [3].

Onion (*Allium cepa* L.), which ranks second globally in terms of consumption among vegetables, possesses a high antioxidant capacity owing to its rich content of bioactive compounds [4]. A member of the Amaryllidaceae family, onion is a plant that has been widely consumed since ancient times as both a food ingredient and an aromatic agent. It is also commonly used in traditional and alternative medicine across various cultures owing to its anti-inflammatory, antimicrobial and antioxidant properties. Onions are particularly rich in vitamins and phytochemical constituents [5, 6]. Chemical composition analyses have revealed that onion consists of approximately 89.1% water, 9.3% carbohydrates, 1.1% protein and 0.1% fat, in addition to various vitamins, minerals and organosulfur compounds that confer its characteristic pungent odour [7]. Analytical studies have identified more than 400 bioactive compounds in onions, including flavonoids, phenolic acids, amino acids, peptides, saponins and fatty acids [8]. This rich and diverse phytochemical profile not only underpins the nutritional and therapeutic potential of onion but also establishes it as a prominent functional food.

Light is one of the fundamental environmental factors governing plant growth and development as it plays a critical role not only in energy-producing processes such as photosynthesis but also in the formation of morphological structures [9]. Recognising this situation has led to efforts to increase the efficiency of using sunlight, our primary natural light source, in production, and to seek alternative light sources. The most important light source in plant production is the sun. Sometimes, weather conditions such as cloud cover and rainfall, as well as seasonal shortening of the day length, prevent plants from meeting their light requirements from the sun. This situation negatively affects plants. Numerous comprehensive studies have been conducted to prevent such light-related problems in production and to increase quality and yield [10]. Furthermore, recent awareness of plant photomorphogenesis and technological developments have led to the need for artificial light sources in plant production. As a result, the use of light-emitting diodes (LEDs), which offer various advantages over traditional light sources, has become widespread [11]. These LEDs are environmentally friendly due to their low power consumption, high light efficiency and low heat output [12]. Furthermore, the fact that these LEDs can be applied at different wavelengths has led to their widespread use, particularly in plant production facilities and plant tissue cultures [13, 14].

Changes in LED wavelengths can affect plant metabolism, leading to alterations in the synthesis of phytochemicals, functional compounds in plants, and in the accumulation of secondary metabolites [15]. Such changes may exert stimulating, inhibiting or suppressive effects on various

physiological processes including growth and development, and consequently induce modifications in the plant's chemical composition both in terms of constituent identity and concentration. In their study on green onion (*Allium fistulosum* L.), Gao et al. [16] found that a white–blue light combination enhanced both morphological development and accumulation of nutritionally valuable components. They also observed that the light spectrum influenced organ-specific volatile compound profiles and increased sulphur-containing compound levels. Similarly, Gao et al. [17] reported that monochromatic and combined light treatments affected growth and development, photosynthetic characteristics and chloroplast structure in onion species. In another study blue LED light supplementation was found to increase antioxidant levels and delay senescence in 'Yuanzang' green onion (*Allium fistulosum* L.), resulting in improvements in flavour, nutritional quality and storage resistance [18].

Similarly, studies conducted on different plant species have shown that high-intensity green LED light promotes growth in lettuce [19], red light increases total phenolic content in lettuce [20], blue LED lighting enhances chlorophyll, carotenoid and vitamin C levels in cabbage [21], white LED lighting is suitable for carotenoid production in cabbage microgreens, blue LED lighting is effective in increasing phenolic accumulation [22], and LED light application influences the concentrations of carotenoid pigments and glucosinolates in spinach [23]. Additionally, studies on various plant species have reported that LED light applied at an appropriate spectral composition can modulate total phenolic compound and flavonoid contents [24-27]. Few studies in the literature have evaluated the effect of LED light on the antioxidant capacity of onion (*Allium cepa* L.). In this context the present study is among the pioneering efforts to examine the effects of LED light applications at different wavelengths on antioxidant compounds during the growth and development of onion. Specifically, the bioactive constituents and antioxidant capacities of leaves from different *A. cepa* L. varieties grown under red, blue, green and white LED lights were analysed.

MATERIALS AND METHODS

Experimental Set-up and Growth Conditions

In this study three onion (*Allium cepa* L.) cultivars (yellow, white and red) were used as plant material. The onion seeds were obtained from certified vendors (red onion: Greal Low 1, İNTFA®, Turkey; yellow onion: Yellow Growe, İNTFA®, Turkey; white onion: Beyazay, İNTFA®, Turkey) authorised by the Turkish Ministry of Agriculture and Forestry. Seeds were sown in pots (three seeds per pot) filled with commercial potting soil (Torflower). Three replicate pots were allocated to each cultivar under each light treatment. The pots were placed in custom-built, light-tight growth chambers (60 × 60 × 60 cm) constructed from expanded polystyrene with TS EN 13163 (European Standard for thermal insulation products made of expanded polystyrene). The chambers were arranged adjacently and featured open fronts to ensure adequate air exchange and thermal regulation while remaining sealed to prevent cross-contamination of light spectra (Figure 1). Each chamber was illuminated by monochromatic or broad-spectrum LEDs mounted on the ceiling, 60 cm above the plant base. The control treatment was equipped with white LEDs (450-660 nm), while experimental treatments was equipped with blue (450 nm), green (490-550 nm) and red (660 nm) LEDs. Light intensity was measured at plant canopy level using a digital lux meter. Photoperiod was maintained at 16 h light / 8 h dark using a programmable digital timer (Table 1). Temperature and relative humidity were kept constant at 22 ± 1°C and 60 ± 5% respectively. Plants were irrigated manually once per week with equal volumes of distilled water (200 ml/pot).

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All chemicals were of analytical grade. Gallic acid, quercetin, folin-ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH•), butylated hydroxytoluene (BHT), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) and neocuproine were purchased from Sigma. UV-visible spectrophotometer (Optima SP-3000, Tokyo/Japan) was used to determine the phenolic and flavonoid contents as well as the anti-radical and reducing power activities of the samples.

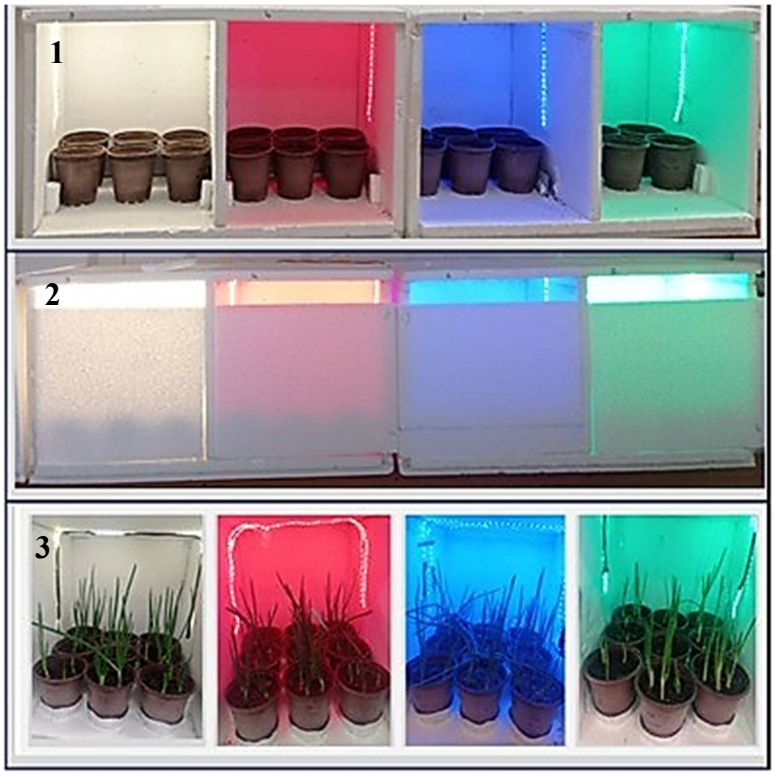

Figure 1. Light cabinets where plants are grown: 1=Onion seed planting period, 2=Onion growing period, 3=Onion harvest period

Table 1. Experimental groups and light treatments

Group	Abbreviation	Cultivar	LED Type (Peak wavelength)	Photoperiod (light/dark)
Control Group	WLI	Red	White (450–660 nm)	16/8 h
Control Group	WLII	Yellow	White (450–660 nm)	16/8 h
Control Group	WLIII	White	White (450–660 nm)	16/8 h
Group 1	BLI	Red	Blue (450 nm)	16/8 h
Group 2	BLII	Yellow	Blue (450 nm)	16/8 h
Group 3	BLIII	White	Blue (450 nm)	16/8 h
Group 4	GLI	Red	Green (490-550 nm)	16/8 h
Group 5	GLII	Yellow	Green (490-550 nm)	16/8 h
Group 6	GLIII	White	Green (490-550 nm)	16/8 h
Group 7	RLI	Red	Red (660 nm)	16/8 h
Group 8	RLII	Yellow	Red (660 nm)	16/8 h
Group 9	RLIII	White	Red (660 nm)	16/8 h

Note: WLI=Red onion grown in white light; WLII=Yellow onion grown in white light; WLIII= White onion grown in white light; BLI=Red onion grown in blue light; BLII=Yellow onion grown in blue light; BLIII=White onion grown in blue light; GLI=Red onion grown in green light; GLII=Yellow onion grown in green light; GLIII=White onion grown in green light; RLI=Red onion grown in red light; RLII=Yellow onion grown in red light; RLIII=White onion grown in red light.

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Preparation of Plant Extracts

The green leafy tissue of onion (*Allium cepa* L.) varieties (red, yellow and white types) grown under LED lights was harvested and used in the study. The harvest was conducted 60 days after seed sowing. Leaf samples were thoroughly cleansed with distilled water to eliminate physical contaminants and excess moisture was removed using paper towels. Subsequently, 3.0 g of fresh leaf tissue from each group were homogenised in 30 mL of 99.8% methanol. The mixture was subjected to overnight agitation, after which it was filtered through Whatman-no.1 filter paper to separate the supernatant [28]. The resulting extract was stored in a refrigerator (4°C) for use in subsequent analyses.

Determination of Phytochemical Properties

Total phenolic content (TPC)

TPC of the extracts was determined using Folin-Ciocalteu colorimetric method [29]. Briefly, 100 µL of each extract was diluted with 1,840 µL of distilled water. Subsequently, 40 µL of Folin-Ciocalteu reagent was added and the mixture was incubated at room temp. for 3 min. Then 120 µL of 2% w/v Na_2CO_3 solution was added. The resulting mixture was then allowed to stand at room temp. for 2 hr. The absorbance of the samples was measured spectrophotometrically at 760 nm against a blank. TPC of the extracts was expressed as mg gallic acid equivalents (GAE)/g of fresh weight using an equation derived from a standard gallic acid curve. All analyses were performed in triplicate.

Total flavonoid content (TFC)

TFC was assessed using aluminium nitrate colorimetric assay [30]. To 100 µL of the leaf extract, 1920 µL of methanol was added. After 1 min., 40 µL of 1 M potassium acetate and 40 µL of 10% (w/v) aluminium nitrate were added sequentially. The mixture was incubated at room temperature for 40 min., and absorbance was measured at 415 nm against a blank (distilled water replacing extract). Results were expressed as mg of quercetin equivalent (QE)/g of fresh weight based on a quercetin standard curve.

DPPH radical scavenging activity

The free radical scavenging capacity of the extracts was evaluated using the DPPH• assay [31]. Extracts were prepared at concentrations of 20, 40, 60 and 80 µg/mL. To 500 µL of each extract solution, 2 mL of 0.1 mM methanolic DPPH• solution was added. The mixtures were incubated in the dark at room temperature for 30 min. and absorbance was measured at 517 nm against methanol. Per cent DPPH• radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH• radical scavenging activity} = [(A_0 - A_1) / A_0] \times 100,$$

where A_0 = absorbance of the control reaction (DPPH• + methanol), A_1 = absorbance of leaf extract/standard solution. The concentration (µg/mL) required to inhibit 50% of DPPH• (IC_{50}) for each group was determined from the concentration–% radical scavenging activity curve.

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Cupric ion reducing antioxidant capacity (CUPRAC) assay

The reducing power of the samples was determined using CUPRAC method with copper(II) ions as oxidant [32]. To each test tube,1 mL of 10 mM copper (II) chloride, 1 mL of 7.5 mM neocuproine and 1 mL of 1 M ammonium acetate were added. Then 0.5 mL of the extract solution prepared at different concentrations (10-50 μg/mL) and 0.5 mL of distilled water were added. After 30 min. of incubation at room temperature in the dark, absorbance was measured at 450 nm.

BHT, Trolox, ascorbic acid and α-tocopherol were used as reference antioxidants. CUPRAC was expressed as Trolox equivalent antioxidant capacity ($TEAC_{CUPRAC}$) by proportioning the slope of the graph drawn using the measurement results with Trolox and the slope of the graph drawn for the sample.

Statistical Analysis

Data were analysed using SPSS version 22 (SPSS Inc., USA). A two-way analysis of variance (ANOVA) was conducted to examine the main effects of light treatment and cultivar, as well as their interaction effect (light×cultivar), on TPC, TFC and antioxidant capacity. In the cases where the differences were significant, Duncan's test, one of the multiple comparison tests, was used to determine which treatment or treatments caused this difference [33]. In addition, $p < 0.05$ was accepted in all calculations in this study.

RESULTS AND DISCUSSION

TPC and TFC of the experimental groups are shown in Table 2. The differences between the groups were found to be significant ($p < 0.05$). The highest amount of phenolic substances was 0.35±0.02 mg GAE/g fresh weight in RLI (red onion grown in red light), and the lowest amount was 0.16±0.03 mg GAE/g fresh weight in GLIII (white onion grown in green light). Saeed et al. reported the phenolic content in onion as 137 ± 1.24 mg GAE/100 g extract in a similar study [34]. Sagar et al. obtained the total phenolic content of 14.55–289.04 mg GAE/g dry weight in a study conducted with 15 varieties of Indian local onions [35]. Kaur et al. reported values of 867.8 mg/kg fresh weight in the red variety, 702.0 mg/kg fresh weight in the pink variety and 165.0 mg/kg fresh weight in the white variety [36]. The values obtained in the present study are consistent with the range of phenolic content reported by Kaur et al. [36].

It is known that phenolic compounds play an important role in the defence mechanisms of plants against pathogen attacks or ultraviolet radiation. It is also reported that the amounts of phenolic compounds in plants may vary according to plant species, variety, climate and cultivation method [37]. In this study since all cultivation parameters were kept uniform across the varieties except for the light spectrum, the observed differences in phenolic contents can be primarily attributed to the genetic characteristics of the onion varieties and their specific physiological responses to the different light colours. A positive correlation was observed between TPC and the colour of onions grown under red LED light, with higher activity in dark-coloured onions. In addition, the TPC in onion plants varied with the colour of light applied during cultivation, and the highest amount was found in plant groups exposed to red light.

In the present study TFC was calculated as QE using a quercetin standard curve and the difference between the groups was found to be significant ($p < 0.05$). The highest flavonoid content was 1.57±0.04 mg QE/g fresh weight in RLI, and the lowest was 0.58±0.05 mg QE/g fresh weight in GLI (red onion grown in green). In another study on onion bulb tissue, flavonoid content was

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reported as 6.98-8.14 µg/g dry weight [38], whereas higher levels are commonly observed in foliar tissues under light supplementation.

Table 2. TPC and TFC of the experimental groups

Group	TPC (mg GAE/g)	TFC (mg QE/g)
RLI	**0.35±0.02^{a}**	**1.57±0.04^{a}**
RLII	0.32±0.01^{b}	1.25±0.08^{b}
RLIII	0.32±0.01^{b}	1.03±0.08^{c}
BLI	0.21±0.06^{g}	0.59±0.01^{g}
BLII	0.28±0.02^{c}	0.69±0.05^{ef}
BLIII	0.26±0.04^{d}	0.75±0.02^{e}
GLI	0.24±0.01^{de}	**0.58±0.05^{g}**
GLII	0.23±0.09^{f}	0.59±0.04^{g}
GLIII	**0.16±0.03^{i}**	0.61±0.02^{fg}
WLI	0.24±0.08^{de}	0.91±0.03^{d}
WLII	0.28±0.07^{c}	0.75±0.05^{e}
WLIII	0.26±0.01^{de}	0.77±0.01^{e}

Note: Differences between means indicated with the same letter are not significant at $p < 0.05$ level.
RLI=Red onion grown in red light; RLII=Yellow onion grown in red light; RLIII=White onion grown in red light; BLI=Red onion grown in blue light; BLII=Yellow onion grown in blue light; BLIII=White onion grown in blue light; GLI=Red onion grown in green light; GLII=Yellow onion grown in green light; GLIII=White onion grown in green light; WLI=Red onion grown in white light; WLII=Yellow onion grown in white light; WLIII=White onion grown in white light.

DPPH• assays were performed and IC_{50} (µg/mL) values for each group are presented in Table 3. Since IC_{50} is inversely related to antioxidant activity, a lower IC_{50} indicates higher radical scavenging capacity. Among the tested groups, the RLII sample (yellow onion grown under red LED) exhibited the lowest IC_{50} value (296.86 ± 10.60 µg/mL), closest to that of the synthetic antioxidant BHT (40.85 ± 5.19 µg/mL), indicating the highest antioxidant activity. Conversely, the BLIII group (white onion grown under blue LED) showed the highest IC_{50} value (578.39 ± 8.11 µg/mL), reflecting the lowest activity. These results suggest that red LED light exerts the strongest effect on antioxidant capacity compared to the control. Previous studies on different onion varieties have reported that radical scavenging activity varies with bulb colour [35, 39]. Similarly, in a study on soybean sprouts grown under different LED lights, highest DPPH• radical scavenging activity was observed in the groups treated with blue LED light compared to the green LED group and the control group [40].

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Table 3. IC_{50} values experimental groups and BHT

Group	IC_{50} (μg/mL)
BHT	40.85 ± 5.19^{i}
RLI	332.15 ± 6.64^{fg}
RLII	$\mathbf{296.86\pm10.60^{h}}$
RLIII	491.24 ± 8.90^{d}
BLI	566.16 ± 16.29^{ab}
BLII	433.66 ± 6.47^{e}
BLIII	578.39 ± 8.11^{a}
GLI	549.99 ± 16.13^{b}
GLII	545.54 ± 5.40^{b}
GLIII	522.14 ± 5.23^{c}
WLI	349.34 ± 10.55^{f}
WLII	314.54 ± 7.47^{gh}
WLIII	505.96 ± 4.08^{cd}

Note: Differences between means indicated with the same letter are not significant at $p < 0.05$ level.
BHT=Butylated hydroxytoluene; RLI=Red onion grown in red light; RLII=Yellow onion grown in red light; RLIII=White onion grown in red light; BLI=Red onion grown in blue light; BLII=Yellow onion grown in blue light; BLIII=White onion grown in blue light; GLI=Red onion grown in green light; GLII=Yellow onion grown in green light; GLIII=White onion grown in green light; WLI=Red onion grown in white light; WLII=Yellow onion grown in white light; WLIII=White onion grown in white light.

Antioxidant capacities were determined using CUPRAC method and the results are presented in Figure 2 and $TEAC_{CUPRAC}$ values are shown in Table 4. In the CUPRAC assay, absorbance at 450 nm increases in parallel with rising extract concentration (10-50 μg/mL), indicating enhanced Cu^{2+} ion reduction capacity. At the highest concentration (50 μg/mL), red onion grown under red LED light exhibits the highest reducing antioxidant power. Among light treatments, onions grown under red light show the greatest reducing capacity whereas those grown under green light display the lowest. Eren [41] reported that green onions possessed higher reducing power than other bulbous plants in his study. When $TEAC_{CUPRAC}$ values of standards and plant samples are compared, the synthetic antioxidant BHT, commonly used as a preservative in foods due to its high antioxidant activity, demonstrates approximately 2.57-fold greater activity than an equivalent amount of Trolox. Among the plant samples, the $TEAC_{CUPRAC}$ value closest to that of Trolox is observed in RLI, while the lowest value is recorded in GLIII. Based on the light colour used during cultivation, the highest $TEAC_{CUPRAC}$ values are obtained in onion varieties grown under red light.

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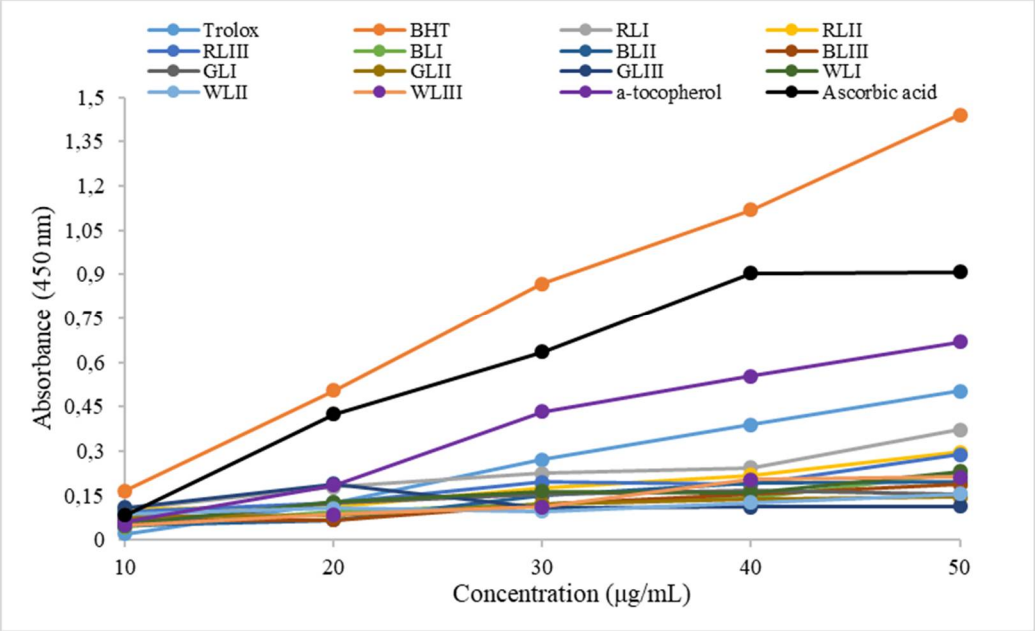

Figure 2. CUPRAC of onion leaf study groups and standards

Note: RLI=Red onion grown in red light; RLII=Yellow onion grown in red light; RLIII=White onion grown in red light; BLI=Red onion grown in blue light; BLII=Yellow onion grown in blue light; BLIII=White onion grown in blue light; GLI=Red onion grown in green light; GLII=Yellow onion grown in green light; GLIII=White onion grown in green light; WLI=Red onion grown in white light; WLII=Yellow onion grown in white light; WLIII=White onion grown in white light.

Table 4. $TEAC_{CUPRAC}$ values for standard and onion leaf samples

Sample	$TEAC_{CUPRAC}$
Trolox	1
BHT	2.5772
α-tocopherol	1.2845
Ascorbic asic	1.7317
RLI	**0.4878**
RLII	0.3983
RLIII	0.3658
BLI	0.3333
BLII	0.3414
BLIII	0.2601
GLI	0.2357
GLII	0.1544
GLIII	**0.0243**
WLI	0.3170
WLII	0.1300
WLIII	0.3577

Note: RLI=Red onion grown in red light; RLII=Yellow onion grown in red light; RLIII=White onion grown in red light; BLI=Red onion grown in blue light; BLII=Yellow onion grown in blue light; BLIII=White onion grown in blue light; GLI=Red onion grown in green light; GLII=Yellow onion grown in green light; GLIII=White onion grown in green light; WLI=Red onion grown in white light; WLII=Yellow onion grown in white light; WLIII=White onion grown in white light.

Light plays an important role in plant physiology, modulation of gene expression, and secondary metabolite biosynthesis [42]. The increase in phenolic compounds under red light can be explained by phytochrome signalling. Red light is detected by the phytochrome B photoreceptor found in plants. Under this light effect, phytochrome B converts from the red light-absorbing form to the far-red light-absorbing form. This conversion initiates a series of signalling events that activate the phenylpropanoid pathway, ultimately leading to an increase in the accumulation of phenolic compounds [43]. The red light response is particularly distinct from blue light effects. While blue light induces phenolics as a stress response, partly via cryptochrome-dependent reactive oxygen species [44], red light acts through a developmental signalling pathway that enhances secondary metabolism without causing oxidative stress [43].

CONCLUSIONS

The phytochemicals of onion plants are affected differently by the different coloured LED treatments applied in the growing environment. In particular, the total amount and antioxidant activities of phytochemicals in onions grown under red light are most strongly influenced. It is therefore suggested that the application of red light to onion plants under cultivation conditions may be more advantageous in terms of phytochemical accumulation than that of other light colours.

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