Effect of light quality on Betalain content of red beet (*Beta vulgaris* L.) cultured *in vitro*

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Background
Red beet (*Beta vulgaris* L.) is the most important edible crop that belongs to the family Amaranthaceae. It is considered as an excellent source of foliate and manganese. It contains betalains, which are considered as natural pigments consisting of two major components, the yellow betaxanthin and violet betacyanin.
Objectives
In this paper, the effect of different light qualities [white, blue, green, red, and ultraviolet (UV)] on the red beet extract content was examined. The main aim of this study was to choose the light quality which will elevate betalains.
Materials and methods
Red beet seeds were well sterilized and germinated on Murashigue and Skoog basal medium. After 4 weeks, the germinated seedlings (explants) were exposed to different wave lengths. Three replicates for each wavelength treatment were collected after 10, 20, and 30 days. Regarding UV treatment, cultures were exposed to UV rays type C for 10, 20, or 30 min, and three replicates for each time period were collected. Fresh weight of each explants was measured and stored at −20°C till further usage. Overall, 0.2 g of fresh weight was used for extraction using 2 ml extraction solvent (80 ml methanol+20-ml sterilized distilled water+100 μl phosphoric acid). The antioxidant activity, total phenols, and betalains were determined using spectrophotometer.
Results and conclusion
The highest values of both fresh weight and free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl percentage were recorded with exposing the cultures to the blue light for 30 days or the exposure to UV rays for 30 min (1.285 and 0.746 g and 42.27 and 43.88%, respectively). It was obviously recorded that exposing the cultures to the red light for 10 days or exposing them to the UV rays for 10 min gave the highest values of the total phenol (1.54 and 0.88 mg GAE/g FW, respectively). The highest value of betalains (Betacyanin and Betaxanthin) was recorded with exposing the cultures to the red light for 30 days (0.12 and 0.077 mg/g FW, respectively).

Keywords:
antioxidant activity, *Beta vulgaris*, betalain content, total phenol content

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Introduction

Red beet (*Beta vulgaris* L.) is the most important edible crop that belongs to the family Amaranthaceae; it includes the former family Chenopodiaceae. It is grown in the temperate zone for its root, which is rich in sucrose content. It is sometimes referred to as beet, which is considered as an excellent source of foliate and a good source of manganese. It contains betalains, which are thought to help control high blood pressure and heart disease. It is considered as a natural pigment that consists of two major component, that is, yellow betaxanthin and violet betacyanin. These tyrosine-derived pigments result from the conjugation of betalamic acid (the chromophore), by means of an imino linkage, with either cyclodopa (which may be glycosylated), giving rise to the betacyanins, or with an amine, resulting in the betaxanthins [1]. Conjugation of the chromophore with either an amine or cyclodopa substituent is believed to occur spontaneously *in vivo* under conditions of acid pH, as found in the plant cell vacuole [2].

Plant biotechnology laboratories can be defined as an agriculture technology where the surrounding microclimate is fully controlled. Light is one of the most important factor in controlled environmental condition, which not only affects plant growth but also affects the production of plant secondary metabolites [3,4]. Plant secondary metabolites are a large number of compounds that do not directly involve in plant growth or development but are required for plant survival. Plant secondary products

are used commonly as drugs, nutraceuticals, and food additives. Owing to their limited availability, an alternative route such as tissue culture is becoming important for large-scale production of these desired compounds [5].

The aim of this paper was to examine the effect of different light quality [white, blue, green, red, and ultraviolet (UV) rays] on the content of the red beet extracts cultured *in vitro*. The characterization of chemical compounds in details is given for red beet extracts by using spectrophotometer to calculate the antioxidant activity, total phenols, and betalains content, that is, yellow betaxanthin and violet betacyanin during different periods of time.

Materials and methods

Plant materials

Red beet (*Beta vulgaris* L.) seeds were well sterilized by washing thoroughly under running tap water for 1 h, and then used 30% Clorox for 10 min, and then washed thoroughly three times by sterilized distilled water. Thereafter, the seeds were germinated on Murashigue and Skoog basal medium.

Effect of exposure to different light quality on the fresh weight (g)

After 4 weeks, the germinated seedlings (explants) were exposed to different wave length; nine replicates were used for each wavelength. Three replicates from each wavelength were collected after 10, 20, and 30 days. For UV treatment, cultures were exposed to UV rays (type C) for 10, 20, or 30 min, with three replicate for each time. Fresh weight of explants was measured, and they were frozen at −20°C till further usage.

Sample extraction

Overall, 200 mg of fresh weight was extracted with 1 ml extraction solvent (80 ml methanol+20 ml sterilized distilled water+100 µl phosphoric acid) for 24 h. Then the sonication for extracts was done in an ultrasonic water bath (Grant, United Kingdom) for 20 min. Samples were centrifuged for 5 min at 6000 rpm (Sigma 2–16 PK; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). The supernatants were collected, and the pellets were re-extracted twice with 1 ml of the solvent according to Kavitha *et al.* [6]. The extracts were stored at −20°C until further use.

Antioxidant activity

The antioxidant activity of each extract was determined through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.

Effect of exposure to different light quality or exposure to ultraviolet ray C on the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The DPPH assay according to Gabr *et al.* [7] was used with some modifications. The stock reagent solution (1×10^{-3} mol/l) was prepared by dissolving 22 mg of DPPH in 50 ml of methanol and stored at −20°C until use. The working solution (6×10^{-5} mol/l) was prepared by mixing 6 ml of stock solution with 100 ml of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm, as measured using a spectrophotometer. Extracts of different samples (0.1 ml of each) were vortexed for 30 s with 3.9 ml of DPPH solution and left to react for 30 min; after which, the absorbance at 515 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

$$\text{Radical scavenging DPPH activity}\,(\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right)\times 100,$$

where A is the absorbance at 515 nm.

Effect of exposure to different light quality or exposure to ultraviolet ray C on the total phenol content (mg GAE/g FW of plants)

Total phenols was determined by the Folin–Ciocalteu micromethod [8,9]. Overall, 20 µl of extract solution was mixed with 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu's reagent, followed by 300 µl of 200 g/l Na_2CO_3 solution. The mixture was incubated in a water bath at 40°C for 30 min, and its absorbance at 760 nm was measured. Gallic acid was used as a standard for the calibration curve. Total phenolic content as gallic acid equivalent was calculated using the following equation:

$$A = 0.98\text{C} + 9.925\times10^{-3}\left(R^2 = 0.9996\right),$$

where A is the absorbance and C is the concentration (mg GAE g^{-1} dry weight).

Effect of exposure to different light quality or exposure to ultraviolet ray C on the betalain content (Betacyanin and Betaxanthin in mg/g fresh weight)

Betacyanin and betaxanthin contents of the extracts were determined spectrophotometrically. Measurements were performed in triplicate, and the betalain content (BC) was calculated according to Cai *et al.* [10], with slight modification. BC (mg/g)=[(A(DF)(MW)Vd/εLWd)] where A is the absorption value at the absorption maximum of 535 and 483 nm for betacyanins and betaxanthins, respectively; DF is the dilution factor; Vd is the dried pulp solution volume (ml); Wd is the dried pulp weight (g); and L is the path-length

(1 cm) of the cuvette. The molecular weight (MW) and molar extinction coefficient (ε) of betanin [MW=550 g/mol; ε=60 000 l/(mol cm) in H_2O] were applied to quantify the betacyanins. Quantitative equivalents of the major betaxanthins (Bx) were determined by applying the mean molar extinction coefficient [ε=48 000 l/(mol cm) in H_2O].

Statistical analysis

All data are presented as mean±SD according to the described method by Snedecor and Cochran [11]. Each mean is the average of three replicates.

Results and discussion

Effect of exposure to the different light quality on the fresh weight (g)

Data presented in Table 1 show the effect of exposure to different light quality (white, red, blue, and green) on fresh weight during 30 days of culturing. Data declared that the fresh weight increased with increasing the exposure periods with all different light quality, as the fresh weight reached its highest values after 30 days of exposing to the different light quality. The highest fresh weight was recorded after 30 days of exposure to the blue light (1.285 g).

Effect of exposure to the ultraviolet rays type C on the fresh weight (g)

Data of Table 2 show the effect of exposure to the UV rays type C for different periods of time (10, 20, and 30 min) on the fresh weight. Data declared that the fresh weight increased gradually with increase in the period of exposure to the UV. It reached its highest value after 30 min of exposing to the UV (0.746 g).

Table 1 Effect of exposure to the different light quality for different periods of time on the fresh weight (in grams)

Treatment	Fresh weight in grams		
	After 10 days	After 20 days	After 30 days
White	0.577±0.01	0.585±0.01	0.628±0.01
Red	0.413±0.008	0.563±0.02	0.788±0.01
Blue	0.327±0.01	0.788±0.01	1.285±0.1
Green	0.204±0.01	0.365±0.03	1.13±0.1

Data are represent as mean±SD.

Table 2 Effect of exposure to the ultraviolet rays type C for different periods of time on the fresh weight (in grams)

Treatments	Fresh weight in grams
Exposure to UV type C for 10 min	0.452±0.01
Exposure to UV type C for 20 min	0.518±0.01
Exposure to UV type C for 30 min	0.746±0.01

Data are represent as mean±SD. UV, ultraviolet.

Effect of exposure to the different light quality on the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

Table 3 shows the effect of exposure to different light quality (white, red, blue, and green) for different periods of time (10, 20, or 30 days) on the DPPH radical scavenging activity % for the extracts. Data obviously declared that the radical scavenging activity % recorded its highest values after 30 days of exposing to the different light quality except with the white one, which recorded its highest value after 20 days of exposing to it and then decreased to reach its lowest value with this light quality. Then the highest radical scavenging activity was recorded after 30 days of exposing to the blue light (42.27%), whereas the lowest value (14.30%) was recorded with exposing to the green light for 10 days.

Effect of exposure to the ultraviolet rays C on the 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity

The effect of exposure to the UV rays type C for different periods of time (10, 20 and 30 min) on the DPPH radical scavenging activity % for the extracts is presented in Table 4. Data obviously declared that the DPPH radical scavenging activity % increased gradually with increase in the exposing period of time to the UV rays. It reached its highest value with 30 min of exposing to the UV (43.88%), whereas the exposure to the UV rays for 10 min gave the lowest value of the DPPH radical scavenging activity % (19.71%).

Table 3 Effect of exposure to the different light quality for different periods of time on the free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl percentage

Treatment	Free radical scavenging capacity of DPPH %		
	After 10 days	After 20 days	After 30 days
White	26.23±3.29	33.64±10.83	23.24±1.28
Red	36.40±6.15	18.29±2.67	38.70±3.40
Blue	37.01±10.80	29.72± 4.62	42.27±2.07
Green	14.30±1.26	31.56±10.13	37.97±2.19

Data are represent as mean±SD. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Table 4 Effect of exposure to the ultra violet rays type C for different periods of time on the free radical scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl percentage

Treatments	Free radical scavenging capacity of DPPH %
Exposure to UV type C for 10 min	19.71±7.24
Exposure to UV type C for 20 min	31.33±15.54
Exposure to UV type C for 30 min	43.88±11.64

Data are represent as mean±SD. DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Effect of exposure to the different light quality on the total phenol content (mgGAE/g FW of plants)

Table 5 shows the effect of exposure to different light quality (white, red, blue, and green) for different periods of time (10, 20, or 30 days) on the total phenol content (mg GAE/g FW of plants) for the extracts. Data obviously reported that the highest total phenol was recorded with exposure of the cultures for 10 days to the red light (1.54), whereas the lowest total phenol content (0.32) was recorded with exposure of the cultures for 10 days to the blue light.

Effect of exposure to the ultraviolet rays C on the total phenol content (mg GAE/g FW of plants)

Data presented in Table 6 show the effect of exposure to the UV rays type C for different periods of time (10, 20, and 30 min) on the total phenol content (mg GAE/g FW of plants) of the extracts. Data showed that the highest total phenol content (0.88) was recorded after 10 min of exposure of the cultures to the UV rays, whereas the lowest total phenol content (0.25) was recorded after 20 min of exposure to the UV rays.

Table 5 Effect of exposure to the different light quality for different periods of time on total phenol content (mg GAE/g FW of plants)

Treatment	Total phenol (mg GAE/g FW of plants)		
	After 10 days	After 20 days	After 30 days
White	0.77±0.02	0.87±0.04	0.33±0.06
Red	1.54±0.05	0.34±0.04	1.13±0.02
Blue	0.32±0.02	0.70±0.03	0.73±0.06
Green	0.33±0.05	0.56±0.04	1.16±0.04

Data are represent as mean±SD.

Effect of exposure to the different light quality on the betalain content (betacyanin and betaxanthin in mg/g fresh weight)

Data of Table 7 show the effect of the exposure to different light quality (white, red, blue, and green) for different periods of time (10, 20, or 30 days) on the betalain content (betacyanin and betaxanthin) of the extracts. Data generally show that the betacyanin and betaxanthin take the same trend with different light and different periods of exposure, as the highest BC (betacyanin and betaxanthin) was recorded after 30 days of exposure to the red light (0.12 and 0.077, respectively), whereas the lowest BC (betacyanin and betaxanthin) was recorded after 30 days of exposure to the blue light (0.013 and 0.016, respectively).

Effect of exposure to the ultraviolet ray C on the betalain content (betacyanin and betaxanthin in mg/g fresh weight)

The effects of exposure to the UV rays type C for different periods of time (10, 20, and 30 min) on the betalain content (betacyanin and betaxanthin) of the extracts were evaluated (Table 8). Data obviously show that the betalain content decreased with increase in the period of exposure to the UV rays. The highest BC (betacyanin and betaxanthin) was recorded after 10 min

Table 6 Effect of exposure to the ultraviolet rays type C for different periods of time on total phenol content (mg GAE/g FW of plants)

Treatments	Total phenol (mg GAE/g FW of plants)
Exposure to UV type C for 10 min	0.88±0.02
Exposure to UV type C for 20 min	0.25±0.05
Exposure to UV type C for 30 min	0.70±0.02

Data are represent as mean±SD. UV, ultraviolet.

Table 7 Effect of exposure to the different light quality for different periods of time on Betalain content (Betacyanin and Betaxanthin in mg/g fresh weight)

Treatments	Betalain content					
	Betacyanin in mg/g FW			Betaxanthin in mg/g FW		
	After 10 days	After 20 days	After 30 days	After 10 days	After 20 days	After 30 days
White	0.023±0.005	0.111±0.044	0.02±0.01	0.025±0.01	0.066±0.02	0.023±0.01
Red	0.094±0.045	0.019±0.001	0.12±0.01	0.065±0.01	0.022±0.01	0.077±0.02
Blue	0.076±0.02	0.02±0.01	0.013±0.002	0.06±0.02	0.021±0.002	0.016±0.001
Green	0.02±0.01	0.06±0.02	0.027±0.01	0.023±0.01	0.042±0.001	0.033±0.003

Data are represent as mean±SD.

Table 8 Effect of exposure to the ultraviolet rays type C for different periods of time on Betalain content (Betacyanin and Betaxanthin in mg/g fresh weight)

Treatments	Betalain content	
	Betacyanin in mg/g FW	Betaxanthin in mg/g FW
Exposure to UV type C for 10 min	0.19±0.02	0.122±0.002
Exposure to U. type C for 20 min	0.083±0.01	0.054±0.002
Exposure to UV type C for 30 min	0.073±0.02	0.047±0.01

Data are represent as mean±SD. UV, ultraviolet.

of exposure of the cultures to the UV rays (0.19 and 0.122 mg/g fresh weight, respectively), whereas the lowest betalain content (betacyanin and betaxanthin) was recorded after 30 min of exposure to the UV rays (0.073 and 0.047 mg/g fresh weight, respectively).

Light is considered the most critical environmental factor that affects plant growth and development [12]. Plants need light for photosynthesis process and also for the regulation of their development. UV and blue radiations are highly involved in different photomorphogenetic effects [13]. Plant growth is a result of some processes like cell division and elongation, directional growth and branching. These processes are affected by light spectrum, intensity, and direction. The sensitivity of plants to light extends from UV, through the visible spectrum to far-red radiation. In the visible light spectrum (400–700 nm), the major wavelengths received by plant photoreceptors and pigments are those belonging to blue (400–500 nm) and red (600–700 nm), and to a lesser extent, green (500–600 nm) [14]. However, Hogewoning *et al.* [15] reported that the blue light is important for photosynthesis process, chlorophyll formation, chloroplast development, and the chemical composition of *Cucumis sativus* plants.

As for growth and fresh weight, our results are in line with that reported by Lee *et al.* [16] who declared that the blue light treatment enhances fresh weight and dry matter production in buckwheat. Moreover, the fresh weight and the dry weight of *Anoectochilus roxburghii*, which was exposed to the blue light treatment, were significantly greater than in the control treatment [17]. On the contrary, it was found that the exposure to UV ray treatment (suitable light intensity and exposure time) increased plant yield in some species according to Sakalauskait *et al.* [18] who declared that exposure for 1 h or 2 h of supplemental UV-B light per day for seven days increased all of plant height, leaf area, fresh weight, and dry weight of sweet basil. As for the DPPH radical scavenging activity, it was declared that the blue light and UV rays have very high frequencies, which in turn mean that they carry a great amount of energy, which may cause damages to the different cellular functions of the plant. To protect the plant tissues from the incoming energy as well as clean up any 'free radicals' produced in the cell, plants will produce different compounds and subsequently increase the DPPH radical scavenging activity [19]. This can be investigated by our results. Moreover, in this respect, Manivannan *et al.* [20] declared that monochromatic red and blue light-emitting diode both enhanced the antioxidant capacities in Chinese foxglove, and blue light was more efficient than red light. In the same time, Kumar *et al.* [21] declared that UV rays can be used in both crop sterilization and can also be used to induce the secondary metabolites production. For the total phenol content, our results take the same trend with that reported with Kliewer [22], who reported that the total content of phenols decreased with increase in the exposure period to the red light in the Emperor grapes. In addition, Shiga *et al.* [23] reported that the amount of rosmarinic acid, which is considered the major component of phenolic compounds in sweet basil under red and white light treatments was double to that under blue light treatment. However, as for betalain content, our results are in contrast to those found by Kishima *et al.* [24] who reported that betalain pigmentation in callus of *Portulaca callus* was induced by blue and blue/UV lights. On the contrary, our results are in line with those found by Ries *et al.* [25] who reported that the red light and white light were used as great inductors for the production of betacyanin in the genus *Alternanthera*.It was thought that betalains were related to anthocyanin. Both betalains and anthocyanin are water-soluble pigments found in plant cell vacuoles. However, betalains are chemically and structurally unlike anthocyanin. and the two will never be found in the same plant. They are not related chemically to the anthocyanin and are not even flavonoids [26].

However, red light has more pronounced effect on the accumulation of anthocyanin than blue light. This can be a result of the increased expression of anthocyanin biosynthesis gene (i.e. MdMYB10 and MdUFGT) under the influence of red light [27].

Conclusion

It is concluded that the exposure to the blue light or the exposure to UV rays has a positive effect on both the fresh weight and on the DPPH radical scavenging activity. However, the red light and UV rays have a positive effect on total phenol content and betalain content. Further research should be conducted. Combinations between different wavelengths could be occurred so as to direct the plant to produce a particular substance or to increase its production.

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Conflicts of interest

There are no conflicts of interest.

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