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Article

Effect of Light Emitting Diode Radiation on Antioxidant Activity of Barley Leaf

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Antioxidant activity of extracts of barley leaves cultivated by light emitting diode (LED) radiation such as red, far-red, blue, blue-red, green, yellow, and white light was investigated. After measuring length and weight of the leaves cultivated, barley leaves were extracted using 70% ethanol. The Hunter color value, total phenolic compounds, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts (ABTS) radical-scavenging activities of extracts were determined. Lengths of samples cultivated by red and green light radiation were 13.7 and 13.6 cm, respectively. Hunter L* values of samples cultivated by red, far-red, and UVA radiation were 65.29, 67.55, and 67.57, respectively. The content of total phenolic compounds of samples cultivated by blue light radiation was 1.62 mg/L of sample. The DPPH radical-scavenging activities of samples cultivated by blue, green, UVA, and white light radiation were 64.28, 48.92, 55.95, and 48.72%, respectively. The ABTS radical-scavenging activity of samples cultivated by blue light radiation scored higher compared with those of samples cultivated with other LED lights. Antioxidant activities of barley leaves showed different results depending on harvest time. Application of LED radiation during re-cultivation after the first harvest showed increasing tendency on antioxidant activity of barley leaves.

Key words: antioxidant activity, barley leaf, harvested time, light emitting diode

The recent abundant evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and diseases has attracted much attention of the scientists and general public to the role of antioxidants in the maintenance of human health, as well as prevention and treatment of diseases [Papas, 1999; Halliwell and Gutteridg, 2007]. Antioxidant may be defined as a substance that, when present at concentration lower than that of the oxidizable substrate, significantly delay or inhibit the oxidation of the substrate [Halliwell, 1997]. The antioxidants function as the first line defense by suppressing the formation of reactive oxygen and nitrogen species (ROS/RNS), by reducing hydrogen peroxide and lipid hydroperoxide to water and lipid hydroxides, respectively, or by sequestering metal ions such as iron and copper [Niki, 2010].

Natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens, and anticarcinogens [Osawa *et al*., 1992]. Among them, barley and its leaf are a good natural source of polyphenol, vitamins, and minerals [Nishlyama *et al*., 1992; Park *et al*., 2008; Lee *et al*., 2010], and also have antioxidant activity in lipid peroxidation system [Nishlyama *et al*., 1992].

However, biological composition in natural plants can vary by cultivar and various environmental factors [Oliveira *et al*., 2007; Kopsell and Kopsell, 2008], among which light is one of the most important variables affecting phytochemical concentrations in plants [Kopsell and Kopsell, 2008; Pérez-Balibrea *et al*., 2008]. It is widely understood that light intensity could positively affect the accumulation of phytochemicals [Vergeer *et al*., 1995]. Moreover, white light-emitting diodes (LED) are considered as the next generation solid-stated lighting systems due to their excellent properties such as high luminous efficiency, energy saving, long lifetime, and lack of toxic mercury [Shen *et al*., 2010].

Recently, several studies to increase biological activity of plant using LED light have been reported. The UV-A

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induction of anthocyanins accumulation was observed in grape [Kataoka *et al*., 2003] and in lettuce [Tsormpatsidis *et al*., 2008]. In addition, the blue (B) light increased the levels of anthocyanins in tomato [Giliberto *et al*., 2005]. The phenolics concentration increased by 6% with supplemental red light, whereas supplemental far-red light decreased anthocyanin, carotenoid, and chlorophyll concentrations of the baby leaf lettuce [Qian and Kubota, 2009]. The objectives of the present study was to investigate the antioxidant activity of barley leaf and the effect of light emitting diode (LED) radiation on the antioxidant activity of barley leaf.

Materials and Methods

Sample preparation and extraction. The covered barley cv. Geungang genetic resource was supplied from Department of Rice and Winter Cereal Crop, National Institute of Crop Science, Korea. To cultivate the barley leaf, 20 g of barley was soaked into the water for 8 h. Soaked barley was planted and cultivated for 6 days by LED radiation including red (660-670 nm), far-red (730-740 nm), blue (470-475 nm), blue-red (470-670 nm), green (505 nm), yellow (590-595 nm), and white (overall wavelengths), as well as UVA, fluorescense light, and dark condition. The samples cultivated under dark condition was placed in a growth chamber without light to achieve the same environmental condition as the LED-radiated samples. Growth chamber was maintained at 20-21°C with 75-80% humidity. After cultivation for 6 days, the sample was harvested (1st harvested sample) and re-cultivated for 4 days under the same condition as the first cultivation. Re-grown barley leaf was harvested (2nd harvested sample) and used. After measuring the length and weight of cultivated barley leaf samples, extraction of the samples was performed using a 70% ethanol solution for 24 h at room temperature. The ratio of sample to solvent was 1:40 (w/v). The extracts were filtered through a 110-mm filter paper (No 2 Advantec Toyo, Tokyo, Japan) and used for further analysis.

Color measurement. Color of the extracts was measured by a color difference meter (Color JS 55; Color Technology System Co., Tokyo, Japan). The color of each sample was measured three times and then averaged. The numerical value of the color was expressed by Hunter L*, a*, and b* values. Hunter L* value indicates the lightness of the samples, a* value indicates the +red/-green, and b* value indicates +yellow/-blue.

Total phenolic contents. Total phenolic contents were measured using the Folin-Ciocalteau colorimetric method [Yu *et al*., 2004]. The extract (0.1 mL) was mixed with 0.2 mL of Folin-Ciocalteau reagent (Sigma Chemical Co., St. Louis, MO), followed by the addition of 3 mL of 5% Na_2CO_3. The absorbance of the mixture at 765 nm was recorded by a spectrophotometer (HP1B; Hewlett-Packard Co., Tokyo, Germany) for the mixtures after 2 h incubation at 23°C. The total phenolic content was expressed as gallic acid equivalents.

Scavenging effects of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH radical-scavenging effect was estimated according to the method of Blois [1958] with some modifications. The sample dissolved in 70% ethanol (1 mL) was added into the 0.2 mM DPPH radical solution (1 mL) and vortexed. The mixture was reacted for 30 min at room temperature, and the absorbance was measured at 517 nm with a spectrophotometer. The scavenging activity of the DPPH radicals in percentage points was calculated by the following equation: Scavenging activity (%)=$(1-A_1/A_0)\times100$, where A_0 is the absorbance of the blank, and A_1 is the absorbance of the sample.

2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts (ABTS) radical cation-scavenging activity. ABTS radical cation was measured using the method of Zhao *et al*. [2006] with some modification. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30°C. The extract (0.1 mL) was mixed with 2.9 mL of diluted $ABTS^{\cdot+}$ solution. After reaction at 30°C for 20 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of $ABTS^{\cdot+}$ scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE)/g.

Statistical analysis. The experiment was designed as randomized block design with three replications. One-way analyses of variance were performed using SAS software (version 7.0; SAS Institute, Cary, NC) along with Duncan's post hoc tests to compare differences among mean values. Each data entry represents the mean of three different experiments with three measurements in each experiment. Mean values and standard errors of the mean (SEM) were reported, and the significance was defined at $p<0.01$.

Results and Discussion

Length and weight of barley leaf cultivated by various LED light radiation. The length and weight of barley leaf cultivated by various LED light radiation are shown in Fig. 1. Length of samples cultivated by red, far-

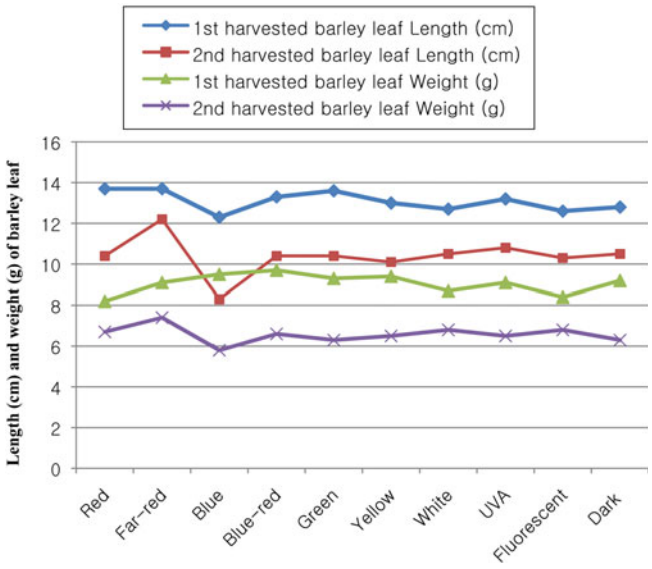

Fig. 1. Length and weight of barley leaves cultivated by various LED radiation.

red, blue, blue-red, green, yellow, UVA, and white light radiation were 13.7, 13.7, 12.3, 13.3, 13.6, 13.0, 13.2, and 12.7 cm, respectively. Length of samples cultivated by red, far-red, and green light radiation showed higher score compared with other radiated samples. Weights of barley leaves cultivated by blue, blue-red, and yellow light radiation were 9.5, 9.7, and 9.4 g, respectively. Lengths and weights of 2nd harvested samples, which were re-cultivated after 1st harvest, showed decreasing tendency as compared with 1st harvested samples. Wu *et al*. [2007] reported that, after radiation for 96 h, compared to white light group, red light-radiated seedling displayed significant (p<0.05) increases in stem length and leaf area, whereas blue light radiation significantly (p<0.05) increased the stem length and seedling weight. Miyashita *et al*. [1995] reported that red and far-red light greatly influence the growth and morphology of potato plantlets. Kim *et al*. [2004] obtained the greatest stem elongation of chrysanthemum plantlet under red and red+far-red LED than fluorescent light, blue, blue+red, and blue+far-red LED.

The Hunter color values of the barley leaf extracts. Changes in the Hunter color value of barley leaf extracts cultivated by LED radiation are shown in Table 1 and Fig. 2. LED radiation during the cultivation induced the color change of the samples. Lightness of samples cultivated by LED radiation including red, far-red, blue, blue-red, green, yellow, white, and UVA light were 65.29, 67.55, 63.79, 64.05, 64.46, 64.81, 64.76, and 67.57, respectively. Lightness of samples cultivated under dark condition was higher than that of the samples cultivated with various LED light. In addition, lightness (L*) of 2nd harvested samples was higher than that of 1st harvested sample extracts. Zhou and Singh [2004] reported that the amount of each individual anthocyanin of cranberry fruit increased differently under natural light, red, and far-red light, suggesting that expressions of enzymes that catalyze the anthocyanin biosynthesis are regulated differently by environments. As revealed by previous studies, blue light is important for chloroplast development, chlorophyll formation, and stomata opening [Senger, 1982; Wu *et al*., 2007]. Our results indicated that lightness of samples cultivated by red, far-red and UVA light radiation scored higher, and redness (+red/-green) of samples cultivated

Table 1. Hunter color value of extracts of barley leaves cultivated by LED light radiation

Treatment	Harvested time					
	1st harvest			2nd harvest		
	L*	a*	b*	L*	a*	b*
Red	65.29^{c}	-32.37^{c}	82.93^{f}	65.85^{h}	-27.77^{c}	54.67^{h}
Far-red	67.55^{b}	-33.39^{d}	85.50^{bcd}	77.19^{b}	-26.30^{b}	63.12^{d}
Blue	63.79^{g}	-32.11^{b}	85.52^{bcd}	65.98^{h}	-32.53^{h}	70.56^{a}
Blue-red	64.05^{f}	-33.66^{e}	85.34^{cd}	66.70^{g}	-31.55^{f}	60.87^{f}
Green	64.46^{e}	-33.37^{d}	86.07^{b}	67.41^{e}	-31.62^{f}	66.11^{c}
Yellow	64.81^{d}	-34.33^{f}	85.13^{d}	67.71^{d}	-28.71^{d}	55.46^{g}
White	64.76^{d}	-33.33^{d}	85.79^{bcd}	67.14^{f}	-32.77^{i}	66.99^{b}
UVA	67.57^{b}	-33.82^{e}	85.86^{bc}	70.55^{c}	-32.17^{g}	62.49^{e}
Fluorescent	62.02^{h}	-32.59^{c}	83.99^{e}	66.79^{g}	-31.43^{e}	66.72^{b}
Dark	83.95^{a}	-13.12^{a}	105.21^{a}	89.90^{a}	-11.89^{a}	35.13^{i}
SEM[1]	0.002	0.202	0.137	0.008	0.011	0.096

[1]Standard error of the mean (n=30).
[a-h]Different letters within the same column with the same sample differ significantly.
[x-y]Different letters within the same row with the same sample differ significantly.

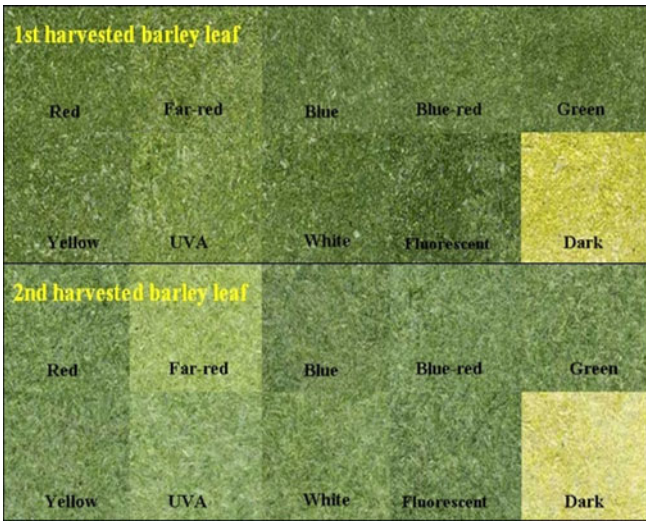

Fig. 2. Color change of barley leaves cultivated by various LED light radiations.

by blue light radiation showed lower value (-32.11) than that of samples cultivated by other LED lights regardless of the harvested time.

Total phenolic compounds. Total phenolic contents of the barley leaf extracts cultivated by red, far-red, blue, blue-red, green, yellow, UVA, and white light radiations were 1.40, 1.46, 1.62, 1.49, 1.53, 1.44, 1.48, and 1.40 mg/L, respectively (Table 2). Blue light radiation during the cultivation of barley leaf was shown to have a positive effect of increasing total phenolic contents in the present study. However, no difference was found in total phenolic contents depending on harvesting time except for samples cultivated by yellow light and dark condition ($p<0.01$). Lee *et al*. [2003] reported that the amount of total phenolic contents in rice hull increased from 0.12 to 0.20 mM after radiation of FIR for 50 min. The higher antioxidant activity coincided with higher phenolic content, and, in the case of light (fluorescent lamp)-germinated corn seedling, the antioxidant activity was high during early growth [Reena *et al*., 2005]. Luthria *et al*. [2006] reported that the phenolic content of tomato fruits is significantly affected by the spectral quality of solar UV radiation.

Antioxidant activity of barley leaf extracts. Antioxidant activity of barley leaf extracts was estimated with DPPH and ABT radical-scavenging activities. The DPPH radical-scavenging activity of barley leaves cultivated by various LED light radiations are shown in Table 3. DPPH radical-scavenging activity of barley leaves showed similar results when compared to total phenolic compound. DPPH radical scavenging activity of samples cultivated by blue LED light radiation and dark condition were 64.28 and 72.34%, respectively, and was significantly higher than those of other samples. Scavenging activity on DPPH radicals of 2nd harvested samples cultivated by red, far-red, blue, blue-red, green, yellow, UVA, and white light radiations were 19.18, 22.02, 33.35, 28.85,

Table 2. Total phenolic compounds (mg/L) of extracts of barley leaves cultivated by LED light radiation

Treatment	Total phenolic compounds (mg/L)		
	Harvested time		
	1st harvest	2nd harvest	SEM[1]
Red	1.40^{b}	1.47^{ab}	0.031
Far-red	1.46^{ab}	1.39^{abc}	0.058
Blue	1.62^{a}	1.51^{a}	0.060
Blue-red	1.49^{ab}	1.48^{ab}	0.063
Green	1.53^{ab}	1.47^{ab}	0.052
Yellow	1.44^{abx}	1.22^{cy}	0.028
White	1.40^{b}	1.27^{bc}	0.077
UVA	1.48^{ab}	1.20^{c}	0.075
Fluorescent	1.44^{ab}	1.28^{bc}	0.078
Dark	1.42^{bx}	1.22^{cy}	0.018
SEM[2]	0.052	0.063	

[1]Standard error of the mean (n=6). [2]Standard error of the mean (n=30).
[a-c]Different letters within the same column with the same sample differ significantly.
[x,y]Different letters within the same row with the same sample differ significantly.

Table 3. DPPH radical-scavenging activity (%) of extracts of barley leaves cultivated by LED light radiation

Treatment	DPPH radical-scavenging activity (%)		
	Harvested time		
	1st harvest	2nd harvest	SEM[1]
Red	36.01^{f}	19.18^{b}	3.248
Far-red	45.85^{de}	22.02^{ab}	4.094
Blue	64.28^{bx}	33.35^{ay}	3.733
Blue-red	44.64^{de}	28.85^{ab}	3.184
Green	48.92^{d}	31.00^{ab}	3.005
Yellow	41.93^{ex}	20.91^{aby}	2.586
White	48.72^{dx}	25.37^{aby}	2.576
UVA	55.95^{cx}	27.08^{aby}	2.779
Fluorescent	48.88^{dx}	26.63^{aby}	0.566
Dark	72.34^{ax}	22.92^{aby}	1.776
SEM[2]	1.754	3.730	

[1]Standard error of the mean (n=6). [2]Standard error of the mean (n=30).
[a-f]Different letters within the same column with the same sample differ significantly.
[x,y]Different letters within the same row with the same sample differ significantly.

Table 4. ABT radical-scavenging activity of extracts of barley leaves cultivated by LED light radiation

Treatment	ABT radical-scavenging activity (μmol TE/g)		
	Harvested time		
	1st harvest	2nd harvest	SEM[1]
Red	1.62^{dy}	1.68^{cx}	0.006
Far-red	1.70^{ab}	1.68^{c}	0.009
Blue	1.66^{bcd}	1.60^{e}	0.034
Blue-red	1.63^{dc}	1.67^{c}	0.007
Green	1.69^{abc}	1.71^{b}	0.006
Yellow	1.73^{ax}	1.68^{cy}	0.007
White	1.62^{dy}	1.72^{bx}	0.007
UVA	1.69^{abcy}	1.73^{ax}	0.001
Fluorescent	1.64^{bcdx}	1.61^{ey}	0.003
Dark	1.69^{abc}	1.65^{d}	0.009
SEM[2]	0.018	0.004	

[1]Standard error of the mean (n=6). [2]Standard error of the mean (n=30).
[a-e]Different letters within the same column with the same sample differ significantly.
[x,y]Different letters within the same row with the same sample differ significantly.

31.00, 20.98, 27.08, and 25.37%, respectively. DPPH radical-scavenging activity of extracts of 2nd harvested samples cultivated by red, far-red, blue-red and green light radiations were not significantly different compared with 1st harvested samples ($p<0.01$), whereas that of the 2nd harvested barley leaf showed increasing tendency by LED radiation compared with samples cultivated under dark condition.

ABT radical-scavenging activity of extracts of 1st harvested barley leaves cultivated by red, far-red, blue, blue-red, green, yellow, UVA, and white light radiations were 1.62, 1.70, 1.66, 1.63, 1.69, 1.73, 1.69, and 1.62 ìmol TE/g, respectively (Table 4). In addition, scavenging activity on ABT radicals of samples cultivated by fluorescent light radiation and dark condition were 1.64 and 1.69 μmol TE/g, respectively. Nam *et al*. [2004] reported that far-infrared radiation significantly increased the antioxidant activity of rice hull extracts, and rice hull extract irradiated by far-infrared had lower TBARS value and fewer volatile aldehydes (hexanal, pentanal, and propanal) than the non-irradiated extract during 3 days of aerobic storage. After far-infrared radiation for 60 min, DPPH radical-scavenging activity of rice hull extracts increased from 47.74 to 81.60%, and this increase was not induced by heat but by the FIR ray [Lee *et al*., 2003]. Wu *et al*. [2007] reported that Trolox equivalent antioxidant capacity (TEAC) of acetone extract from pea seedling under red LED light was the greatest (81.68 μM) and significantly ($p<0.05$) higher than those of white light group (71.61 μM), blue light group (46.49 μM), and dark group (17.47 μM). Thus, application of proper LED light is necessary to meet different purposes. Detailed studies are required regarding the application of LED light for seedling growth in terms of economic utility, nutrition enhancement, and the correlation between light quality and growth of dietary seedlings [Wu *et al*., 2007]. In conclusion, results of the present study indicated that antioxidant activity of extracts of barley leaves were affected by LED light source and harvest time. LED radiation such as blue and green light showed positive effect on antioxidant activity of barley leaf extracts. Radical-scavenging activity on DPPH and ABT of 2nd harvested samples showed decreasing tendency compared with 1st harvested samples. However, LED radiation during the re-cultivation after 1st harvest showed increasing tendency on antioxidant activity of barley leaf compared with cultivated samples under dark condition.

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