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Responses of phenolic acid and flavonoid synthesis to blue and blue-violet light depends on plant species

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Graphical abstract

Plant Species	Importance of Supplemental Light		Response
	+B	+BV	
A			n/a
B			Phenolic Acids ↑
C			Flavonoids ↑

Highlights

- Phenolic acid and flavonoid production of three common culinary herbs show different responses to supplemental blue (+B) and blue-violet (+BV) lights.
- No responses were observed in *Rumex sanguineus*.
- Enhanced production of phenolic acids in *Ocimum basilicum* occurred in response to both light supplements.
- Enhanced production of flavonoids in *Eruca sativa* occurred in response to both light supplements, but strongly under +BV.

Abstract

Three common culinary herbs were studied in order to find out how manipulation of blue range of wavelength spectrum affects plant growth and chemistry. The studied species were basil (*Ocimum basilicum*), arugula (*Eruca sativa*) and bloody dock (*Rumex sanguineus*). It was hypothesized that high-energy short wavelengths induce stress and further increase phytochemical production for light screening. The study was arranged in a greenhouse, where traditional high-pressure sodium lamps served as control treatment. The other two treatments were supplemental blue light (+B) (max. peak at 450 nm) and supplemental blue-violet (+BV) light (max. peaks at 420 and 440 nm). LED lights were used to produce these supplementations. Generally light treatments did not induce marked stress as evaluated by chlorophyll fluorescence ratio (Fv/Fm). However, all growth parameters (shoot elongation, leaf biomass, leaf number) tended to be highest in control treatment, except for leaf number in arugula. Phytochemical production of bloody dock was responsive to neither blue light treatments (+B and +BV). Instead of that, both blue light treatments increased production of phenolic acids in basil, and flavonoids in arugula. In arugula, +BV was even more efficient, indicating the role of flavonoids in protection against UV-A radiation in this species. The role of blue to red light ratio is discussed in relation to quantitative expression in phytochemical synthesis.

Key words: blue light; blue-violet light; growth; flavonoids; phenolic acids; stress

1. Introduction

LED lighting has recently received lots of attention among agricultural and horticultural scientists (e.g. Dueck et al. 2016), not the least due to many practical reasons and applications. Recently, Taulavuori et al. (2017) reviewed many beneficial properties of LEDs compared to conventional greenhouse lights (e.g. luminous efficiency, life span, low electricity use, low heat emissions etc.), highlighting the overall eco-friendly nature of LEDs. The review on 24 research articles suggests that LED lighting efficiently provides additional value for sustainable plant cultivation, since targeted use of LEDs may enrich phytochemicals markedly.

Phytochemicals are natural compounds usually synthesized by plant secondary metabolism. One of the major phytochemical group is comprised of phenolic compounds and their derivatives. At least 24 research papers published during 2013-2017 document that artificial blue light (400-500 nm), supplied by LEDs, may markedly boost the phytochemical production (Taulavuori et al. 2017). For

example, concentration of a flavonoid hyperin increased 4-fold in tomato under supplemental blue (Taulavuori et al. 2013). Phytochemical production, however, is a relatively complex process, since many factors may affect the accumulation of compounds. First, phytochemical production is dependent on plant species (Taulavuori et al. 2016). Some species produce naturally high concentrations of certain compounds, like cichoric acid as a major phenolic compound in lettuce (Rajashejkar et al. 2012; Becker et al. 2013). Second, at least light intensity (e.g. Oh et al. 2009) and especially light spectrum (i.e. light quality) (e.g. Li and Kubota 2009) are environmental factors that strongly modify the magnitude of phytochemical production.

In addition to plant chemistry, light quality modifies plant architecture and morphology, the phenomenon known as photomorphogenesis (Cashmore et al. 2006). Blue light, for example, significantly reduces shoot elongation of Scots pine (Taulavuori et al. 2005; Sarala et al. 2007) and increases compactness of tree seedlings (Riikonen et al. 2016). Suggested mechanism is a gene expression based reduction in cell wall extensibility, which is not necessarily related to availability of photosynthetic products (Huché-Thélier et al. 2016). Photosynthetic activity increases as a function of blue light level, given that other wavelengths are also present (e.g. Hogewoning et al. 2010; Hernandez and Kubota 2014).

Light is fundamentally electromagnetic radiation with specific energy, which is inversely proportional to wavelength: lower the wavelength, higher the energy. Short wavelength ultraviolet radiation ranges from 280 nm to 400 nm, above which blue wavelengths of the light spectrum exist (400-500 nm). UV radiation causes oxidative stress, which may damage cellular structures such as DNA, proteins, lipids and photosynthetic machinery (e.g. Rozema et al. 1997). It was recently shown that also blue light may induce oxidative burst in *Arabidopsis* (El-Esawi et al. 2017). Therefore, it may be expected that proportional increase in short wavelengths in the light spectrum also results in increasing stress in plant tissue. Consequently, variable chemical compounds (flavonoids, phenolic acids, anthocyanins and carotenoids) should be accumulated to attenuate the energy rich wavelengths (Searles et al. 2001; Kotilainen et al. 2008; Li and Kubota 2009; Huché-Thélier et al. 2016, and references therein). Such production of secondary compounds may further result in growth–defense tradeoffs (e.g. Huot et al. 2014).

Aim of the work was to study responses in culinary herbs to two blue light supplemental spectra (blue vs. blue-violet), differing in peak distributions. We hypothesized that both spectra cause stress and impair growth, and that the spectrum containing violet tone (i.e. max. peaks at < 450 nm) is more detrimental compared to exclusively pure blue light (i.e. max. peak at 450 nm). Finally, we expected also species-specific responses.

2. Materials and methods

2.1. Experimental design and arrangements

The experiment was conducted in a greenhouse at the Botanical Gardens of University of Oulu (65°N) in a period from 5th Sept to 23rd Nov 2015. Three different species, basil (*Ocimum basilicum*), arugula (*Eruca sativa*) and bloody dock (*Rumex sanguineus*) were chosen as study plants for their wide use as culinary herbs, known for their aromatic tastes. Bloody dock was especially chosen for its colouring as well as historical background as a medicinal plant. The source of all seeds was Siemenvesa Ltd. (Pori, Finland). The cultivar of basil was ‘Genoverser’, while the other two species had no specific cultivar. Seeds (2 – 4 per pot) were sowed 10th Sep in pots (7 x 7 cm in size) containing slightly fertilized commercial sand-peat sowing mixture (Kekkilä, W HS R8017, NPK 15-5-24). Seeds germinated and pre-grew at +19 °C in the greenhouse of Botanical Gardens for one month. Only one seedling that appeared most vigor was left to grow in each pot.

At the beginning of October (5th Oct) the seedlings were transferred to the experimental greenhouse under the HPS and LED lamps. The experiment consisted of three different light treatments: (1) Control light provided by high pressure sodium lamp (HPS) (400 W, max at 600 nm); the control also served as background light in providing red wavelengths for two supplemental lights, i.e. (2) blue (+B, max peaks at 450 nm) and (3) blue-violet (+BV, max peaks at 420 and 440 nm) treatments. 120 W LED (light emitting diode) panels (Led Finland Ltd.), which located in interspace between HPS lamps and plants, generated the supplemental lights. Detailed spectra of the treatments are shown in Fig. 1. Photosynthetically active radiation (PAR) was measured with Li-Cor-6400. PAR was set to $300 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all treatments by adjusting the elevation of each lamp. The outdoor solar radiation increased the amount of PAR slightly (approx. $10 \mu\text{mol m}^{-2} \text{s}^{-1}$) during noon hours at the beginning of experiment, but fibre cloths hung on the sun-faced walls removed the problem. Room

temperature was set to +19°C, RH 60 % and the lights were on continuously during experiment. The 24 h photoperiod was used to eliminate possible interactions between light quality and daylength as the focus here was in light quality effects. Pots were kept moist by regular irrigation at three day intervals.

Each light treatment had five replicate plots ($n = 5$), each containing 8 individuals of each plant species making total amount of plants 24 per replicate. In practice, these 24 seedlings in each replicate were randomly distributed in two shallow boxes (i.e. sub-plots). This arrangement allowed us to rotate plants 90° clockwise in every third day to expose each side of the plot to equal illumination as in Taulavuori et al. (2016).

2.2. Sampling schedule

Shoot elongation and chlorophyll fluorescence (Fv/Fm) were measured approx. at 10-day intervals. Only last measurements on each species before termination of the experiment were included in the presented data. Each species had different length of elongation period under the treatments, and after the growth had levelled off the final measurements were taken on days 32 (6th Nov), 39 (13th Nov), and 50 (23th Nov) for arugula, basil and bloody dock, respectively. Photosynthesis was measured after Fv/Fm and shoot elongation measurements, followed by counting of leaf numbers and analyses of leaf biomass per replicate plot. Phytochemicals were analyzed from dried (at 60 °C) and powdered leaf total biomass. Otherwise, the samples of the fully expanded leaves were randomly chosen from the upper part plant.

2.3. Measurements and analyses

Chlorophyll fluorescence ratio (Fv/Fm) of leaves was measured with PAM 2000 (Walz Ltd.) portable fluorometer. One leaf from top of each plant species per treatment was randomly chosen ($n= 5$). Prior to measurements, the detached leaves were dark-adapted for 15 min. The length of plant aerial part was measured with a ruler at 1 mm accuracy. Because of the different growth forms, it was performed as follows: In basil the length was measured from the bottom of stem to the tip meristem, and in arugula and bloody dock to the tip of the longest leaf of rosette.

The net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured with Li-Cor-6400 under HPS lamps at 300 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ light intensity and at +20°C temperature. Carbon dioxide (approx. 380 ppm) was taken in the photosynthesis measurement from neighbor greenhouse room via 10 m long silicone tube. Photosynthesis was allowed to stabilize first under black plastic bag for dark respiration level, which was followed by removal of bag and consequent start of photosynthesis. Photosynthesis was followed for 3 min period, recording the rates at 15 s interval which were later averaged for one measurement. Once leaf number was counted, the leaves of each replicate per species were collected into their own paper bag. The leaves were dried at +60°C for 4 days. Dried leaves were grinded with a mill into a fine powder. The phenolic acids and flavonoids were further extracted (8 - 10 mg of powder) and analyzed by HPLC as described previously (Taulavuori et al. 2013; 2016).

Statistical comparison between the treatments was carried out by one-way ANOVA (IBM SPSS Statistics 20 Software). Games-Howell comparison was employed in the cases of unequal variances. Otherwise, post hoc comparison was performed with Sheffe's test.

3. Results

Supplemental blue (+B) and blue-violet (+BV) light neither had significant effect on stress (F_v/F_m), photosynthesis or shoot elongation in basil (Tab. 1). Marginal ($P < 0.1$) effects, however, were found in biomass accumulation and number of leaves, indicating a trend that these were highest in controls and lowest in +BV. Stress, shoot elongation and biomass of arugula were also unaffected by the light supplements, while +BV increased photosynthesis ($P < 0.05$), and +B increased number of leaves ($P < 0.05$) in this species. In bloody dock, +BV reduced shoot elongation ($P < 0.05$) and amount of biomass ($P < 0.05$). Also a trend ($P < 0.1$) appeared according to which the number of leaves was reduced due to +B and +BV treatments. Concerning all species in parallel, the following trends were observable: Light treatments did not cause stress and all growth parameters tended to be highest in control treatment, except for leaf number in arugula.

Phytochemical profiles (Tab. 2) varied markedly between the studied species, where 12 phytochemicals were identified in arugula, and seven and six compounds were detected in basil and bloody dock, respectively. The main result was that +B or +BV, or both, light treatments enhanced many phytochemicals in basil and arugula compared to control light treatment. Bloody dock did not

increase phytochemical production significantly, but many compounds, nevertheless, had the lowest concentrations in control plants. In basil, *p*-OH-cinnamic acid, chlorogenic acid derivative 2 and cichoric acid concentrations increased significantly ($P < 0.05$) in plants of both blue light treatments. Isorhamnetin-diclycoside and luteolin-glycoside derivatives increased ($P < 0.05$) by both +B and +BV treatments in arugula. Both treatments also increased marginally ($P < 0.1$) isorhamnetin-glycoside concentration. +BV alone increased apigenin derivative 1 ($P < 0.01$) and derivative 2 ($P < 0.05$).

4. Discussion

Chlorophyll fluorescence ratio (Fv/Fm) that describes photochemical efficiency of PSII, is considered as universal tool to indicate any stress in plants (e.g. Lichtenthaler 1996; Maxwell and Johnson 2000). Given this fact, our data reveal no stress state in the studied plants under blue or blue-violet treatments of the experiment (Tab. 1). Therefore, the finding is partly against our first hypothesis. However, concerning growth parameters (shoot elongation, biomass, number of leaves), some stress due to light treatments may be acknowledged. This argument can be justified by the best growth characteristics occurred generally in all species under control treatment. Indeed, here the Fv/Fm ratios of basil were in a range of 0.8 – 0.6, while in our previous study (Taulavuori et al. 2016) the ratios were slightly lower (0.7 - 0.5), indicating some stress. Fig. 1 shows that blue (B) and red (R) light were supplied in 1:1 ratio in the present work, which may not be very stressful for basil. In our previous work with lower Fv/Fm, the B:R ratio was twice as high (2:1) (Taulavuori et al. 2016). This is in line with our hypothesis according to which higher energy containing blue light may cause stress, and consistently increasing B:R ratio is also reported to decrease growth of lettuce (Son and Oh 2013). Moreover, elongation of cucumber seedlings decreases when proportion of blue gradually increases until B:R 75:25, while with 100% B the elongation is significantly highest (Hernández and Kubota 2016). Similar trend is reported for height growth of Scots pine seedlings, while increased proportion of B does not reduce the elongation of Norway spruce (Riikonen et al. 2016).

While significant in arugula only, photosynthesis was generally lowest in control plants of all species. This is in accordance with reports that blue light increases photosynthetic activity in the presence of other wavelengths (e.g. Hogewoning et al. 2010; Hernandez and Kubota 2014). Improved photosynthesis nevertheless did not increase growth in the present experiment. However, it seems

that carbon was allocated in phytochemical accumulation instead of vegetative growth, as the overall phytochemical production was generally highest in plants under blue light treatments. Concerning basil especially, comparison of the results with previous work (Taulavuori et al. 2013) also supports this suggestion: Elongation of plants was much lower (9-12 cm) than in the present work (25-28 cm). In parallel with poor growth, some degree of stress and higher concentration of bioactive compounds occurred in the previous work (Taulavuori et al. 2013), which is in agreement with growth–defense tradeoffs (e.g. Huot et al. 2014).

Certain plant species are naturally inducible for specific phytochemicals (e.g. Taulavuori et al. 2016). Basil, for example, synthesized only few compounds including chlorogenic acid, cichoric acid, feruloyl tartaric acid and rosmarinic acid in accordance with previous studies (Taulavuori et al. 2013; 2016). Especially rosmarinic acid is the major phenolic compound in basil as also indicated previously (Jaysinghe et al. 2003; Lee and Scagel 2009; Taulavuori et al. 2013; 2016). In agreement with Bell et al. (2015), the present study shows that arugula is rich in kaempferol-related compounds. Moreover, pool of the phenolic compounds in arugula is relatively wide, indicated by the 12 detected flavonoids. In comparison, only few phenolic compounds were detected in bloody dock (5 compounds) and in basil (7 compounds) in the present study. These five compounds in bloody dock belong to flavonoids, while the all seven compounds in basil belong to phenolic acids.

The main absorption of phenolic compounds occurs in wavelengths of ultraviolet radiation (Searles et al. 2001; Kotilainen et al. 2008) and thus many flavonoids increase markedly in response to enhanced UVB radiation (e.g. Ghasemzadeh et al. 2016). Nevertheless, flavonoids also are major blue light absorbing pigments in a range 400-430 nm (Gitelson et al. 2017). In the longer wavelengths, the light absorption by flavonoids decreases markedly in parallel with increasing absorbance by anthocyanins. The visible red color of bloody dock refers to high anthocyanin concentration. Given that light treatments had no effect on detected phytochemical concentrations of bloody dock, and that flavonoids are precursors of anthocyanins, one may speculate that some of flavonoids metabolized to anthocyanins in this naturally anthocyanin self-productive species. Visual observations also speak for the enhanced anthocyanin production in bloody dock, since interveinal areas of leaf blade tended to become reddish under + BV (Fig. 2), which contained also small proportion of UV-A (i.e. 315 – 400 nm) radiation with the left peak close 400 nm (Fig. 1). On the other hand, green colored leaves of

arugula without any visible signs of excessive anthocyanin synthesis, were most responsive to blue light treatments (especially the +BV) in accumulation of flavonoids.

Flavonoid and phenolic acid concentrations may vary within same species. For example, chicoric acid concentration/content/amount in the present work was much lower (0.26 mg g⁻¹, dw) than in our two previous studies (1.3 mg g⁻¹, dw in Taulavuori et al. 2013; 6.65 mg g⁻¹, dw in Taulavuori et al. 2016). Affecting factors may be cultivar of species (Nguyen et al 2010) or seed source and accession (Javanmardi et al 2002). According to Flanigan and Niemeyer (2014), cultivar has no effect on total phenol content in basil, but it modifies contents of individual phenolic acids. Many environmental factors such as water deficit, nutrients and temperature may affect the concentrations, and also variation between plant structures occurs (e.g. Lee and Scagel 2013, and references therein). Postharvest conditions such as drying may affect the results: Kim et al. (2000) reported about accelerated loss of chicoric acid in *E. purpurea* flowers when dried at 70°C instead of 40 and 25°C. This could explain the lower phenolic acid and flavonoid concentrations in the present work compared to our previous studies (Taulavuori et al. 2013; 2016): In these two latter studies leaves were dried at room temperature, while in the present study drying temperature was +60°C. In agreement, temperature above 60°C generally is considered too high for phytochemical extraction (e.g. Julkunen-Tiitto 1985). This is also supported by findings according to which oven-drying decreases flavonoid concentrations in *Salix* sp. (Julkunen-Tiitto and Sorsa 2001). However, high temperature (i.e. + 80°C) may not necessarily affect non-flavonoid phenolic concentration while phenolic glycosides level may be even higher at + 80°C due to their thermostability properties (Keinänen and Julkunen-Tiitto 1996). We suggest that drying issue needs further investigation, e.g. for species or tissue specificity (woody vs. herbaceous).

In conclusions, blue light induces phenolic acid and flavonoid biosynthesis in plants, and that the responses are species-specific. However, the present study also provided also novel information on the species-specificity, as bloody dock did not respond to either of supplemental blue light. In turn, supplemental blue light increased phytochemicals in both, basil and arugula, still with couple of species-specific responses. First, the specific phenolics produced by basil were phenolic acids, while arugula increased synthesis of flavonoid compounds. Second, the phenolic acids in basil were independent of quality of blue light (+B vs. +BV), while violet containing blue light (+BV) was somewhat more effective in stimulation of flavonoid synthesis in arugula. The results are applicable in manipulation of culinary herbs, for example. Enhancing specific areas of light spectrum is in

essential role in manipulation of desired responses, while specific wavelength ratios (e.g. B:R) in the spectrum should be considered as well.

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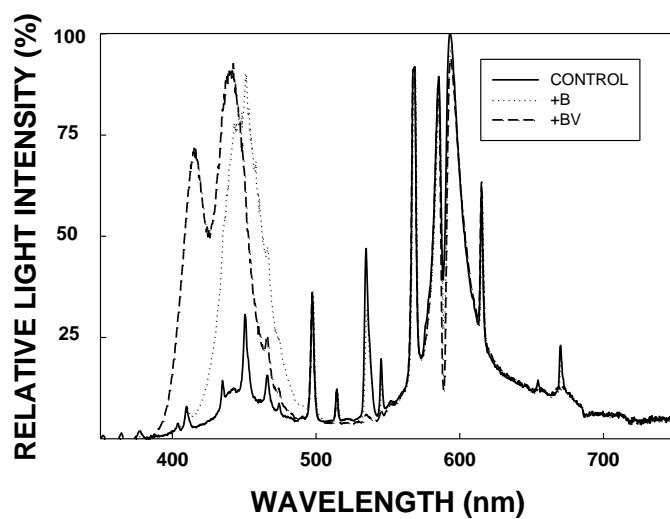


Figure 1. Spectra of control, +B and +BV treatments. NOTE: +BV continues to UV-A range (i.e. to 380 nm)



Figure 2. Colouration of leaf blade in bloody dock under +BV treatment, indicating increased anthocyanin synthesis.

Table 1. Mean values (\pm SE, $n = 5$ replicate plots) and ANOVA results for physiological (Fv/Fm, photosynthesis) and morphological (shoot elongation, biomass, number of leaves) variables of basil, arugula and bloody dock in treatments C (control), +B (supplemental blue) and +BV (supplemental blue-violet) light. Different letters (a-c) indicate subsets after posthoc comparison at $P < 0.05$.

SPECIES	COMPOUND	TREATMENT			ANOVA	
		C	+B	+BV	sig.	df F
Basil	Fv/Fm	0.81 \pm 0.01	0.79 \pm 0.01	0.61 \pm 0.13	NS	2 2.13
	Photosynthesis (μ mol m ⁻² s ⁻¹)	0.98 \pm 0.14	1.26 \pm 0.30	0.93 \pm 0.14	NS	2 0.76
	Shoot elongation (mm)	276 \pm 9	257 \pm 16	248 \pm 17	NS	2 0.96
	Biomass (g, DW)	11.5 \pm 0.52	11.5 \pm 0.43	9.5 \pm 0.85	(P < 0.1)	2 3.15
	Number of leaves	29.4 \pm 1.75	24.9 \pm 1.22	22.38 \pm 2.56	(P < 0.1)	2 3.43
	Arugula	Fv/Fm	0.79 \pm 0.02	0.80 \pm 0.02	0.77 \pm 0.04	NS
Photosynthesis (μ mol m ⁻² s ⁻¹)		0.26 \pm 0.14 a	0.55 \pm 0.16 ab	1.04 \pm 0.20 b	P < 0.05	2 5.71
Shoot elongation (mm)		113 \pm 9.5	111 \pm 6.7	99 \pm 10.0	NS	2 0.68
Biomass (g, DW)		0.37 \pm 0.02	0.28 \pm 0.11	0.21 \pm 0.07	NS	2 1.16
Number of leaves		6.9 \pm 0.42	8.73 \pm 0.56	6.1 \pm 0.88	P < 0.05	2 4.19
Bloody dock		Fv/Fm	0.82 \pm 0.00	0.79 \pm 0.03	0.82 \pm 0.01	NS
	Photosynthesis (μ mol m ⁻² s ⁻¹)	1.23 \pm 0.25	1.04 \pm 0.13	1.56 \pm 0.14	NS	2 1.93
	Shoot elongation (mm)	194 \pm 2.4 b	177 \pm 3.8 a	180 \pm 5.0 ab	P < 0.05	2 5.05
	Biomass (g, DW)	1.49 \pm 0.08 b	1.19 \pm 0.01 a	1.35 \pm 0.08 ab	P < 0.05	2 5.16
	Number of leaves	20.6 \pm 0.78	18.3 \pm 0.89	18.3 \pm 1.22	(P < 0.1)	2 3.54

Table 2. Mean values (\pm SE, $n = 5$ replicate plots) and ANOVA results for phytochemicals (mg g⁻¹, dw) of basil, arugula and bloody dock in treatments C (control), +B (supplemental blue) and +BV (supplemental blue-violet) light. Different letters (a-c) indicate subsets after posthoc comparison at $P < 0.05$. Asterisks (*) behind significance indicate Games-Howell comparison in the cases with unequal homogeneity of variances.

SPECIES	COMPOUND	TREATMENTS			sig.	ANOVA	
		C	+B	+BV		d	F
Basil	Chlorogenic acid	0.032 \pm 0.004	0.053 \pm 0.004	0.049 \pm 0.008	NS	2	2.44
	Chlorogenic acid derivative 1	0.129 \pm 0.007	0.158 \pm 0.008	0.127 \pm 0.009	NS	2	0.16
	p-OH-cinnamic acid	0.015 \pm 0.002	0.021 \pm 0.002	0.020 \pm 0.001	$P < 0.05$	2	4.44
	Chlorogenic acid derivative 2	0.160 \pm 0.010 a	0.261 \pm 0.007 b	0.026 \pm 0.003 b	$P < 0.01$	2	7.81
	Cichoric acid	0.261 \pm 0.012 a	0.503 \pm 0.007 b	0.508 \pm 0.006 b	$P < 0.01$	2	8.24
	Feruloyl tartaric acid	0.043 \pm 0.006	0.067 \pm 0.004	0.054 \pm 0.000	NS	2	1.42
	Rosmarinic acid	1.002 \pm 0.106	1.952 \pm 0.074	2.107 \pm 0.463	NS	2	2.71
	Kaempferol derivative (triglycoside)	0.237 \pm 0.017	0.228 \pm 0.005	0.309 \pm 0.002	NS	2	2.17
	Myricetin-diglycoside	0.059 \pm 0.005	0.076 \pm 0.002	0.092 \pm 0.002	NS $P < 0.05$	2	19.7
Arugula	Isorhamnetin-diglycoside	0.000 \pm 0.000 a	0.048 \pm 0.007 b	0.110 \pm 0.003 b	*	2	7
	Kaempferol-diglycoside	8.362 \pm 0.586	9.223 \pm 0.634	10.500 \pm 0.798	NS	2	2.51
	Luteolin-glycoside derivative 1	0.363 \pm 0.077 a	0.701 \pm 0.05 b	0.713 \pm 0.06 b	$P < 0.05$	2	6.81
	Apigenin derivative 1	0.027 \pm 0.006 a	0.040 \pm 0.006 ab	0.056 \pm 0.004 b	$P < 0.01$	2	8.21
	Apigenin derivative 2	0.060 \pm 0.007 a	0.079 \pm 0.007 ab	0.097 \pm 0.005 b	$P < 0.05$	2	5.27
	Luteolin-glycoside derivative 2	0.000 \pm 0.000 a	0.021 \pm 0.005 b	0.036 \pm 0.005 b	0.05 *	2	20.6
	Hyperin	0.051 \pm 0.010	0.236 \pm 0.006	0.262 \pm 0.013	NS	2	1.75
	Kaempferol glycoside	0.089 \pm 0.029	0.052 \pm 0.001	0.097 \pm 0.009	NS	2	1.33
	Isorhamnetin-glycoside	0.053 \pm 0.017	0.231 \pm 0.006	0.224 \pm 0.007	$(P < 0.1)$	2	3.02

	Apigenin derivative	3	0.129±0.0	0.110±0.01	0.108±0.00	NS	2	1.13
		11		5	3	<i>Games-Howell comparison*</i>		
Bloody dock	Gallic acid	06	0.126±0.0	0.128±0.02	0.115±0.01	NS	2	0.18
	(+)-Catechin derivative	14	0.114±0.0	0.120±0.03	0.089±0.03	NS	2	0.32
	(+)-Catechin	32	0.338±0.0	0.449±0.14	0.365±0.15	NS	2	0.22
	Quercetin-3-glucuronide	80	0.786±0.0	1.094±0.25	1.073±0.31	NS	2	0.52
	Kampferol-glycoside	04	0.031±0.0	0.054±0.01	0.053±0.01	NS	2	0.88
	Kampferol 3-glucuronide	20	0.196±0.0	0.407±0.06	0.461±0.16	NS	2	1.89