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Short-term UV-B radiation effects on morphology, physiological traits and accumulation of bioactive compounds in *Prunella vulgaris* L.

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ABSTRACT

Prunella vulgaris plants (full-bloom stage) were subjected to short-term (15 days) UV-B radiation in a growth chamber. UV-B radiation was effective at enhancing morphological and biomass characteristics and decreasing chlorophyll contents of *P. vulgaris*. Treatment of *P. vulgaris* with artificial UV-B radiation significantly increased peroxidase (POD), superoxide dismutase (SOD) and glutathione (GSH) activities compared to the control treatment. UV-B radiation significantly increased the levels of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and proline in leaves of *P. vulgaris* compared to those of control plants. In addition, the contents of total flavonoids, rosmarinic acid, caffeic acid and hyperoside significantly increased under UV-B radiation. The total phenolic levels also increased under UV-B treatment. These results demonstrated that short-term UV-B radiation can enhance production of secondary metabolites in *P. vulgaris*, resulting in increased spica yield compared to that of control plants.

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Prunella vulgaris L.; UV-B radiation; morphology; antioxidants; secondary metabolite

Introduction

In recent years, depletion of the stratospheric ozone layer because of atmospheric pollutant sources such as bromine- and chlorine-containing compounds has led to an increase in ultraviolet-B radiation (UV-B) reaching the earth's surface (Bjorn 1996; McKenzie et al. 2003). In general, several studies have indicated the harmful influence of UV-B radiation, leading to leaf discoloration, plant stunting and reductions in leaf area, leaf number, biomass and plant productivity (Frohnmeyer and Staiger 2003).

Previous studies have reported that UV-B radiation is a serious environmental stress that enhances the generation of reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hydroxyl radicals (OH) (Mackerness et al. 1998; He and Hader 2002). Plants respond to oxidative damage by activating their antioxidant metabolism, including activation of the enzymes superoxide dismutase (SOD) and peroxidase (POD), which scavenge ROS and offer protection to nucleic acids, lipids and proteins (Jain et al. 2004; Zu et al. 2010). Plants can also produce UV-B-absorbing compounds, such as phenolics and flavonoids, in order to protect from UV-B radiation damage (Tegelberg et al. 2001). Although UV-B-absorbing compounds mainly protect the DNA (Stapleton 1992), these compounds also play a key role in the plant antioxidative defense system and against herbivores and pathogens (Ortuñoa et al. 2006; Hagen et al. 2007; Agrawal et al. 2009).

Prunella vulgaris L. (Labiatae) is commonly known as an important medicinal plant that is typically found in Europe and Northeast Asia (Chen et al. 2011, 2013). The *P. vulgaris* dried spica, Prunellae Spica (called 'Xiakucao' in Chinese), is a standard medicinal material in the Chinese pharmacopoeia (Pharmacopoeia of the People's Republic of China 2015) and is occasionally used for the treatment of thyroid gland malfunction and of mastitis; for detumescence; and as a sedative and an antifebrile agent (Huang et al. 2013). Prunellae Spica is rich in phenolic acids. Rosmarinic acid and caffeic acid as the major phenolic components in Prunellae Spica, exhibits a wide spectrum of biological activities, such as superoxide radical scavenging, antioxidant, anti-fungal and anti-inflammatory effects (Psotová et al. 2005; Chen et al. 2009; Liu et al. 2009; Chen et al. 2013). Meanwhile, flavonoids in Prunellae Spica with significant antioxidant activities and superoxide radical scavenging (Zhang et al. 2011). In addition to their pharmaceutical uses, the dried spicas are used to produce functional beverages, and the leaves of the seedling stage are consumed as vegetables in South China (Chen et al. 2012a, 2012b; Yu et al. 2016).

Previous studies had found that *P. vulgaris* exposed to abiotic factors such as drought (Chen et al. 2011), heavy metals (Wu et al. 2010) and different soil nutrient concentrations (Chen et al. 2013) exhibits significantly increased contents of the major bioactive components (e.g. rosmarinic acid) in plants. The authors suggested that abiotic stresses such as drought, soil nutrient deficiency and heavy metals trigger ROS generation; in turn, the ROS may act as a mediator in initiating biosynthesis of certain secondary metabolites (Shohael et al. 2006; Pu et al. 2009). Despite ROS being a potent inducer, UV-B radiation is rarely if

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ever investigated to determine whether or not it can enhance the biosynthesis of secondary metabolites in *P. vulgaris*.

Therefore, we aimed to compare the morphological traits, photosynthetic pigment contents, antioxidative defense system (e.g. SOD, POD and GSH) and the production of malondialdehyde (MDA), proline, H_2O_2 and secondary metabolites (e.g. total phenolics, total flavonoids, rosmarinic acid, caffeic acid and hyperoside) in plants subjected to short-term UV-B radiation.

Materials and methods

Plant material and growth conditions

A pot experiment was carried out under natural conditions at the College of Pharmaceutical Sciences at Chengdu Medical College, Chengdu, PR China. *P. vulgaris* seeds were collected in June 2015 from the experimental planting station at Chengdu Medical College, Sichuan Province and sterilized with 9% H_2O_2 for 30 min followed by rinsing several times with distilled water, after which the seeds were sown into plastic pots (17 cm diameter and 12 cm height) containing 400 g of nutrient-rich soil. Twenty seeds of similar size were sown into each pot on 21 October 2015. After emergence, which occurred 15 days after planting, all pots were moved into growth chambers with 60–70% relative humidity, 800 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR), and 22°C/14°C (day/night) air temperatures. After one month, the seedlings were thinned to contain three seedlings per pot and watered three times a week. All pots were maintained in growth chambers for six months.

UV treatments

On 5 May 2016, all pots were randomly divided into two treatments. One treatment was subjected to natural light (control), and the other treatment was subjected to UV-B radiation. Each pot contained three seedlings, each treatment had 12 replicates (pots). And each treatment consisted of 36 plants. UV-B treatments were provided daily for 30 min from 20:00 to 20:30 for 15 days in growth chambers. The UV-B radiation was artificially provided by three fluorescent lamps (Beijing Research Institute of Electric Light Source, 40 W, China). The lamps were fixed on a movable frame over the plant canopy, and the distance between the plants and the lamps was approximately 25 cm. The UV fluorescent lamps were wrapped with 0.125-mm-thick diacetate cellulose film (Lucky Films Co. Ltd., Hebei, China) to filter out the UV-C irradiation (<280 nm) in the UV-B treatments. The UV-B irradiance at the top of the plant canopy under the lamps was measured with a portable light meter (UV-340A, Lutron, Taiwan). The UV-B dose for the UV-B treated plants was 35 μW cm^{-2} nm^{-1}, while the control plants received 0.00 μW cm^{-2} nm^{-1}. Films were changed every three days to avoid spectral transmission of UV radiation that could have led to plant aging. On 20 May 2016 (after 15 days of UV-B treatment), four sets of treated and control plants were harvested to determine the photosynthetic pigment and biochemical traits. On 30 May 2016 (10 days after the end of the UV-B treatment), another eight sets of treated and control plants were also harvested to investigate the plant growth indicators and secondary metabolite production.

Plant growth parameters

Plant growth parameters including spica length, spica width, number of branches, and number of spica were measured. The spica length and width were determined with a Vernier caliper. The whole plants were divided into different organs (leaves, stem, spica and roots), dried at 70°C for 12 h, and then measured using an electronic balance (JJ124BC, Electronic Balance Test Instrument Factory, Changshu, China).

Photosynthetic pigment contents

Fresh leaf tissue (0.2 g) was cut into pieces and extracted in 15 mL of 95% ethanol and then placed in darkness and shook a few times for 24 h at room temperature (approximately 25°C). The chlorophyll a and b (chl a and chl b) and carotenoids (car) contents were measured with a 752 UV-visible spectrophotometer (Shanghai Jinghua Technological Instrument, Shanghai, China). The absorbance of the supernatant was recorded at three different wavelengths (470, 648 and 664 nm). Each treatment was repeated three times. The pigment concentrations were expressed in terms of milligrams per gram of FW and calculated using equations and extinction coefficients according to the methods of Chen et al. (2016).

Antioxidant enzyme activities

Fresh leaf parts (0.5 g) were ground in liquid nitrogen and then suspended in a 4.0-mL solution containing 0.1 mol L^{-1} phosphate buffer (pH 7.33). The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatant was collected to determine the activities of peroxidase (POD) and SOD using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All enzyme activities in the test were measured using a 752 UV-visible spectrophotometer (Shanghai Jinghua Technological Instrument, Shanghai, China). Five plants provided sufficient amounts of fresh leaf tissues for use in each experimental replicate ($n = 3$).

The POD activity was measured based on the change of absorbance at 420 nm by catalyzing H_2O_2. One unit of POD activity was defined as 1 milligram of substrate catalyzing 0.01 micromole of H_2O_2 for 1 min in the reaction system at 37°C. The POD activity was reported as units per milligram of protein.

The SOD activity was determined by measuring the rate of enzymatic ability to inhibit O_2^-·morpholine with xanthine oxidase using a SOD assay kit. Each endpoint assay detected the red substances of the reaction system by absorbance at 550 nm after 40 min of reaction time at 37°C. One unit (U) of SOD activity was defined as the quantity of SOD required to inhibit 50% of the reduction of nitrite (1 mL) in reaction solution by measuring the change in absorbance at 550 nm. The SOD activity was reported as units per milligram of protein.

The reduced glutathione (GSH) was measured using an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Fresh leaf tissue (0.5 g) was prepared for homogenization with a 0.1 mol L^{-1} phosphate buffer (pH 7.33). Homogenates were then centrifuged at 12,000 rpm (at 4°C) for 10 min to collect the supernatant for determination of GSH concentrations. The GSH activity was measured based

on the change of absorbance at 420 nm. The GSH content was reported milligrams per gram of protein.

H_2O_2 content

The H_2O_2 content was measured using an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance of a peroxo-polymolybdic acid solution was determined at 405 nm. The H_2O_2 content was reported as millimoles per gram of protein.

MDA and proline contents

The MDA content was determined using an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Briefly, a pestled homogenate of phosphate-buffered saline (PBS) was used for centrifuging fresh leaves (0.5 g) for 10 min at 5000 rpm. The supernatant was added to thiobarbituric acid (TBA) in a boiling water bath for 10 min. The supernatant was then obtained after centrifuging for 10 min at 3500 rpm, and its absorbance was measured at 532 nm. The MDA content was expressed as nanomoles per milligram of protein.

The proline content was determined by the colorimetric method of a proline assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance of proline was measured at 520 nm. The proline content was expressed as micrograms per gram of FW.

Total phenolic contents

The powder of dried spica (1.0 g) was mixed with 10 mL of 80% ethanol at 90°C in a water bath and stirred for 1.5 h. The total phenolic content was determined by the modified methods of Fazal et al. (2016). Briefly, 0.2 mL of the aforementioned extracted solution was diluted with 4 mL of 70% ethanol. In addition, 2 mL of sodium dodecyl sulfate (0.3%) and 1 mL of a mixture solution (0.6% ferric trichloride ($FeCl_3$) and 0.9% potassium ferricyanide $K_3[Fe(CN)_6]$ at a ratio of 1:0.9) were added to the solution. Then, 0.1 mol L^{-1} HCl solution was added to the solution to reach a final volume of 25 mL, and the final solution was then incubated for 20 min in darkness. Afterward, the absorbance was determined at 720 nm using a 752 UV-visible spectrophotometer (Shanghai Jinghua Technological Instrument, Shanghai, China). Caffeic acid was used to generate the standard calibration curve, and the results were expressed as milligrams of caffeic acid equivalents per 100 mg of spica dry weight (%).

Total flavonoid contents

The total flavonoid contents were determined using the colorimetric method with modifications (Fazal et al. 2016). The dried spica powder (1.0 g) was extracted with 10 mL of 35% ethanol at 86°C in a water bath and stirred for 3.5 h. One milliliter of the abovementioned extract solution was mixed with 0.7 mL of 50 mg mL^{-1} $NaNO_3$ in a test tube for 7 min. Then, 0.3 mL of 100 mg mL^{-1} $AlCl_3$ was added to the solution, and the mixture was incubated for 6 min before adding 5.0 mL of 1 mol L^{-1} NaOH solution. The absorbance was measured at 510 nm using a 752 UV-visible spectrophotometer (Shanghai Jinghua Technological Instrument, Shanghai, China). Rutin was used to generate a standard calibration curve, and the results were expressed as milligrams of rutin equivalents per 100 mg of spica dry weight (%).

Rosmarinic acid, caffeic acid and hyperoside

Dried spica powder (2 g) was mixed with 15 mL of 80% methanol in an ultrasonic bath at ambient temperature for 35 min, and then the extracted solution was centrifuged at 12,000 rpm for 15 min. The supernatant was passed through a 0.45-μm organic membrane filter before HPLC analysis. The extract (10 μL) was analyzed on a Dionex UltiMate 3000 HPLC System (Dionex Corp., Sunnyvale, CA, USA) equipped with a Uranus C18 column (250 mm× 4.6 mm) and a diode array detector (DAD-3000). The mobile phase consisted of methanol (solvent A) and 0.2% NaH_2PO_4 solution (solvent B), a flow rate of 0.8 mL min^{-1}, a column oven temperature of 30°C, and a run time of 60 min. The gradient elution used was as follows: 0–20 min, solution A 20–40%; 20–35 min, solution A 40–70%; 35–45 min, solution A 70–90%; and 45–60 min, solution A 90–20%. Detections were carried out at wavelengths of 325 for both rosmarinic acid and caffeic acid and at 360 nm for hyperoside. The identification of each peak was based on the retention time and the chromatography of the authentic standards. The concentrations of phenolic and flavonoid compounds were calculated according to calibration curves of the standards, and the results were represented as milligrams per 100 mg of spica dry weight (%).

Statistical analyses

Data were presented as the means ± SD ($n \geq 3$) and were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS 17.0 software (SPSS, Chicago, IL, USA).

Results

Morphology and biomass

The morphological and biomass characteristics of *P. vulgaris* were measured under UV-B treatment (Table 1). The spica width, stem weight, spica weight and whole-plant weight significantly increased due to UV-B radiation. The spica number per plant, branch number per plant, root weight and leaf weight increased under the influence of UV-B radiation, but these differences were not significant (Table 1). The spica length also decreased due to UV-B radiation, but the difference was not significant.

Table 1. The influence of UV-B radiation on the morphological and biomass traits of *P. vulgaris*.

Characteristics	Control	UV-B
Spica length (cm)	5.44 ± 0.56 a	5.23 ± 1.20 a
Spica width (cm)	1.26 ± 0.12 a	1.41 ± 0.12 b
Spica number per plant	7.0 ± 1.05 a	7.8 ± 1.40 a
Branch number per plant	6.0 ± 1.01 a	6.8 ± 1.40 a
Root weight (g $plant^{-1}$)	0.31 ± 0.09 a	0.42 ± 0.15 a
Stem weight (g $plant^{-1}$)	3.84 ± 0.61 a	5.19 ± 1.32 b
Leaf weight (g $plant^{-1}$)	1.39 ± 0.52 a	1.50 ± 0.54 a
Spica weight (g $plant^{-1}$)	2.33 ± 0.35 a	3.10 ± 0.74 b
Whole-plant weight (g $plant^{-1}$)	8.03 ± 1.14 a	10.66 ± 2.33 b

Notes: Each value is presented as the mean ± SD ($n = 10$). Different letters in a line are significantly different at $p < .05$.

Photosynthetic pigments

The contents of photosynthetic pigments of *P. vulgaris* under UV-B radiation were measured (Table 2). The concentrations of chlorophyll a and total chlorophyll were significantly reduced by UV-B radiation. The concentrations of chlorophyll b and carotenoids decreased under UV-B radiation, but the differences were not significant.

Antioxidant systems

The activities of SOD and POD and the GSH content were measured under UV-B radiation (Table 3). The activity of POD increased under UV-B radiation, but the difference was not significant. In contrast, the SOD activity and GSH content increased significantly under UV-B radiation.

Contents of H_2O_2, MDA and proline

The contents of H_2O_2, MDA and proline were measured under UV-B radiation (Table 4). The contents of H_2O_2, MDA and proline subjected to the UV-B radiation markedly increased by 64.81%, 485.92% and 42.67%, respectively, compared with those of the control treatment.

Contents of secondary metabolites

The contents of secondary metabolites were measured under UV-B radiation (Table 5). UV-B radiation significantly enhanced the contents of total flavonoids, rosmarinic acid, caffeic acid and hyperoside in the spicas of *P. vulgaris* compared with the control treatment. And the content of total phenolics was increased by UV-B radiation, but no significant difference was observed between the two treatments.

Table 2. The influence of UV-B radiation on the chlorophyll contents in leaves of *P. vulgaris*.

Pigment parameter	Control	UV-B
Chlorophyll a (mg g^{-1})	0.62 ± 0.02 a	0.51 ± 0.03 b
Chlorophyll b (mg g^{-1})	0.31 ± 0.01 a	0.27 ± 0.02 a
Total chlorophyll (mg g^{-1})	0.92 ± 0.03 a	0.77 ± 0.05 b
Carotenoids (mg g^{-1})	0.09 ± 0.01 a	0.08 ± 0.01 a

Notes: Each value is presented as the mean ± SD ($n = 3$). Different letters in a line are significantly different at $p < .05$.

Table 3. The effects of UV-B radiation on the activities of SOD and POD and on the GSH content in leaves of *P. vulgaris*.

Antioxidant system	Control	UV-B
SOD activity (U mg^{-1} of protein)	5.51 ± 0.02 a	10.21 ± 0.13 b
POD activity (U mg^{-1} of protein)	37.69 ± 13.57 a	47.08 ± 5.11 a
GSH content (mg g^{-1} of protein)	105.81 ± 2.13 a	121.30 ± 3.44 b

Notes: Each value is presented as the mean ± SD ($n = 3$). Different letters in a line are significantly different at $p < .05$.

Table 4. The influence of UV-B radiation on the H_2O_2, MDA, and proline contents in *P. vulgaris* leaves.

Oxidative stress indicators	Control	UV-B
H_2O_2 (mmol g^{-1} of protein)	93.67 ± 0.64 a	154.38 ± 5.38 b
MDA (nmol mg^{-1} of protein)	1.42 ± 0.27 a	8.32 ± 0.26 b
Proline (μg g^{-1} of FW)	18.02 ± 0.25 a	25.71 ± 0.58 b

Notes: Each value is presented as the mean ± SD ($n = 3$). Different letters in a line are significantly different at $p < .05$.

Table 5. The influence of UV-B radiation on the contents of total phenolics, total flavonoids, rosmarinic acid, caffeic acid and hyperoside in spicas of *P. vulgaris*.

Secondary metabolites	Control	UV-B
Total phenolics (%)	1.38 ± 0.13 a	1.64 ± 0.20 a
Total flavonoids (%)	3.82 ± 0.11 a	7.93 ± 0.46 b
Rosmarinic acid (%)	0.43 ± 0.00 a	0.66 ± 0.00 b
Caffeic acid (%)	0.031 ± 0.000 a	0.038 ± 0.000 b
Hyperoside (%)	0.019 ± 0.000 a	0.042 ± 0.000 b

Notes: Each value is presented as the mean ± SD ($n = 3$). Different letters in a line are significantly different at $p < .05$.

Discussion

In the present study, the influence of UV-B radiation on the morphology, physiological characteristics and secondary metabolites of *P. vulgaris* were investigated. Previous studies indicated that increased UV-B radiation promotes or inhibits plant growth (Day et al. 1999; Zu et al. 2010; Rai et al. 2011) and that the impact of UV-B radiation on plant growth appeared to be species-specific (Deckmyn and Impens 1998). In our study, significant increases in spica width, stem weight, spica weight and whole-plant weight as well as slight increases in spica number, branch number, root weight and leaf weight (except for a slight decrease in spica length) were observed, showing that *P. vulgaris* plants could address the potential hazards by counteracting the effects of UV-B radiation. Similar results have been reported with other medicinal plants subjected to UV-B radiation (Zu et al. 2010; Rai et al. 2011).

Previous studies have reported positive, neutral and negative influences of UV-B radiation on chlorophyll content in plants. The response of chlorophyll levels to increased UV-B radiation depends on environmental conditions and on the development phase of plants (Day et al. 2002; Zu et al. 2010; Rai et al. 2011). Our study indicated that chlorophyll a and total chlorophyll significantly decreased and that chlorophyll b also decreased under UV-B radiation, which may lead to reduced photosynthesis caused by a decline in the ribulose-1,5-bisphosphate carboxylase (Rubisco) protein (Kadur et al. 2007). Carotenoids protect chlorophyll molecules against photooxidative damage and are valid scavengers of ROS that result from UV-B irradiation (Rai et al. 2011). Most studies have shown that the levels of carotenoids increase due to UV-B radiation (Sangtarash et al. 2009), but these levels also have been reported to decrease under UV-B radiation (Steel and Keller 2000; Zu et al. 2010). Our results suggest that the carotenoid content decreased with increased UV-B radiation, perhaps because the pathway that increased the induction of carotenoids caused by UV-B may have already been saturated (Deckmyn et al. 1994; Zu et al. 2010). This result is consistent with those of previous studies, which reported that the levels of carotenoids were decreased under UV-B radiation (Musil et al. 1999; Zu et al. 2010). Our research showed that the carotenoids levels were reduced by UV-B radiation, the mechanism need to be further researched.

The enzymatic and non-enzymatic antioxidants in plants can provide adequate protection against ROS and free radicals induced by photooxidative damage under UV-B radiation (Rai et al. 2011; Takshak and Agrawal 2015). In the present study, SOD and POD activities and the GSH content increased in leaves of *P. vulgaris* subjected to UV-B radiation. A similar study reported markedly greater SOD, POD and ascorbate peroxidase (APX) activities after UV-B exposure

in leaves of *Artemisia annua* L. (Rai et al. 2011). In addition, another study reported that the GSH content increased significantly in rice and Arabidopsis thaliana under UV-B irradiation (Wu et al. 2001; Gao 2007). It is well known that SOD accelerates the conversion of superoxide to hydrogen peroxide and that POD and CAT catalyze H_2O_2 into H_2O and O_2 (Gill and Tuteja 2010). GSH is also an indicator of oxidative stress, and GSH can protect plant cells from oxidative damage and react directly with ROS (Aravind and Prasad 2005). Therefore, the increase in SOD and POD activities and GSH content during UV-B exposure could be to eliminate the ROS production induced by photooxidative stress.

UV-B radiation induces ROS formation and oxidative damage in some medicinal plants (Rai et al. 2011). Higher H_2O_2 content was induced by UV-B radiation than by the control treatment, which implies photooxidative damage (Fedina et al. 2006; Takshak and Agrawal 2015). MDA can be an indicator of lipid peroxidation in medicinal plants subjected to UV-B radiation (Rai et al. 2011; Takshak and Agrawal 2015). In our study, the MDA content in leaves of *P. vulgaris* increased significantly under UV-B radiation, indicating membrane damage. This result is in agreement with those of earlier reports (Zhao et al. 2003; Rai et al. 2011). In addition, proline is directly involved in the reactions of ROS and in the protection of plants against UV-B radiation (Fedina et al. 2006). Our study showed that the increased proline content from UV-B exposure may offer *P. vulgaris* plants protection against ROS generated by the UV-B radiation. Similar results were observed in previous studies (Katerova et al. 2009; Rai et al. 2011).

Phenolic compounds are important secondary metabolites in spicas of *P. vulgaris* (Chen et al. 2013), and the antioxidative activities of phenolic extracts from *P. vulgaris* plants in experimental models suppress lipoperoxidation (Škottová et al. 2004) and scavenge ROS (Osakabe et al. 2004). In this study, the total phenolic contents in spicas of *P. vulgaris* under UV-B radiation were slightly higher than those of control treatment. Similar results were obtained by Manaf et al. (2016) and Inostroza-Blancheteau et al. (2014). Many studies have shown that phenolic compounds absorb in the UV-B electromagnetic region (280–320 nm) that and these compounds function as ROS scavengers to protect plants against UV-B radiation (Tegelberg et al. 2001; Ortuñoa et al. 2006). This result suggested that increasing the total phenolic contents of *P. vulgaris* could have beneficial effects on scavenging ROS induced by UV-B radiation.

Flavonoids are important secondary metabolites in *P. vulgaris* (Chen et al. 2013), and the flavonoids in spicas of *P. vulgaris* have significant antioxidant activities in in vivo experiments (Zhang et al. 2011). These bioactive components were effective at scavenging ROS, and these compounds could be directly committed to increased photoprotection against UV-B radiation (Bors et al. 1990). At the same time, flavonoids could help maintain photosynthetic pigments levels and normal photosynthetic activity (Day and Neale 2002). In the present study, flavonoid contents in the spicas of *P. vulgaris* clearly increased under UV-B radiation, which is consistent with reports in previous studies (Sun et al. 2010; Zu et al. 2010). Thus, our study indicated that increased flavonoids of *P. vulgaris* could protect the plants against UV-B radiation.

Rosmarinic acid is one of the most important secondary metabolites in *P. vulgaris* spicas (Chen et al. 2013). The Chinese Pharmacopoeia (2015) considers the amount of rosmarinic acid as a quality control marker of Prunellae Spica. In this study, UV-B radiation significantly promoted rosmarinic acid accumulation in *P. vulgaris* spicas; this phenomenon was also observed by Luis et al. (2007). Previous studies showed that rosmarinic acid has excellent ROS-scavenging capabilities (Nuytinck et al. 1986). Pharmacological studies indicated that rosmarinic acid significantly inhibits UV radiation-induced ROS generation in human skin cells (Psotova et al. 2006; Sánchez-Campillo et al. 2009). Therefore, the increase in rosmarinic acid could play an important role in protecting *P. vulgaris* plants against ROS production induced by UV-B radiation.

Caffeic acid is an important secondary metabolite in *P. vulgaris* spicas (Chen et al. 2013). Extracts from *P. vulgaris* spicas that contain major amounts of caffeic acid showed that its antioxidative activities may be responsible for the antinociceptive and antidiabetogenic properties in diabetic mice models (Raafat et al. 2016). In this experiment, the caffeic acid content in *P. vulgaris* spicas significantly increased under UV-B radiation, which is consistent with reports of Luis et al. (2007) and Manaf et al. (2016). Previous studies have reported that caffeic acid has strong photoprotective activity against UV-B radiation in both in vivo and in vitro experiments (Saija et al. 2000). Similar studies have shown that the antioxidant activity of caffeic acid can protect phospholipidic biomembranes from UV-B-induced peroxidation (Saija et al. 2000; Prasad et al. 2009). Hence, caffeic acid may prevent lipid peroxidation and ROS damage in *P. vulgaris* plant cells. This protective ability may be the reason for increased caffeic acid content observed in plants under UV-B radiation.

Hyperoside is a major bioactive compound in *P. vulgaris* spicas (Kim et al. 2014) and exhibits a variety of biological activities, such as anticancer, anti-inflammatory, antioxidant and cardiovascular protective effects (Choi et al. 2011; Kim et al. 2011; Huo et al. 2014; Liu et al. 2016). In this study, UV-B radiation increased hyperoside accumulation in *P. vulgaris* spicas. A similar result was observed by Dang (2015). Hyperoside is an effective ROS scavenger, and the highest DPPH-scavenging capability was reported by Liu et al. (2012). Hyperoside was also found to show potent hepatoprotective activity by enhancing the antioxidative defense system against liver injuries induced by CCl_4 (Choi et al. 2011). Therefore, increased hyperoside of *P. vulgaris* spicas may act as compensators to scavenge ROS generated from UV-B radiation.

Conclusions

The results demonstrate that 35 $\mu W\ cm^{-2}\ nm^{-1}$ of UV-B radiation was effective at enhancing morphological and biomass characteristics (except for spica length) and decreasing chlorophyll contents of *P. vulgaris*. UV-B irradiation induced the accumulation of ROS, resulting in higher membrane lipid peroxidation. At the same time, UV-B irradiation promoted enzymes and non-enzymatic systems to eliminate ROS in *P. vulgaris*. In addition, the UV-B-absorbing compounds rosmarinic acid, caffeic acid and hyperoside of *P. vulgaris* spicas played vital roles in protecting plants against UV-B radiation. Our results indicated that UV-B radiation led to a significant increase in the synthesis of bioactive compounds and a higher spica yield in *P. vulgaris*. However, detailed mechanisms of

the genes involved in the regulation of secondary metabolites under UV-B radiation remain to be investigated.

Disclosure statement

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