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VARIATION IN ANTIOXIDANT ACTIVITIES AND BIOACTIVE COMPONENT CONTENTS OF *SALVIA PRZEWALSKII* MAXIM GROWN IN CHINA

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Abstract

Salvia przewalskii Maxim is an abundant and endemic plant in China, and many of its secondary metabolites are important antioxidants. In this study, the antioxidant property of the leaf and root extracts and the contents of eight main bioactive components, namely, salvianolic acids A and B, rosmarinic acid, protocatechualdehyde, tanshinone IIA, tanshinone I, cryptotanshinone, and dihydrotanshinone I, in the leaf and root extracts were evaluated. Results provide scientific guidance on the efficient utilization of *S. przewalskii* as a source of natural antioxidants. The bioactive component concentrations and antioxidant activities of leaf and root extracts remarkably varied with season, and all extracts showed considerable antioxidant activity. Except for the superoxide anion radical-scavenging activity during the flowering stage, the root extract exhibited stronger antioxidant activity than the leaf extract in the same stage, which were mainly contributed by high hydrophilic component contents, such as rosmarinic acid, protocatechualdehyde, and salvianolic acid B. The correlation analysis results indicated that protocatechualdehyde was remarkably associated with 1,1-diphenyl-2-picrylhydrazide (DPPH) free radical-scavenging activity. Rosmarinic acid was notably associated with reducing power and DPPH and superoxide anion radical-scavenging activities. Salvianolic acid B was considerably associated with reducing power and DPPH free radical-scavenging activity. Therefore, given the high hydrophilic component contents, *S. przewalskii* leaves collected from the flowering period could also be used as very powerful herbal antioxidants, which also benefits the recycling of agricultural waste.

Key words: *Salvia przewalskii* Maxim; Bioactive components; Antioxidant activities; High performance liquid chromatography.

Introduction

Oxidative stress is related to various diseases (Yu *et al*., 2013); thus, antioxidants are important to scientists and the public (Kalleem, 2014). Antioxidants not only exhibit great importance in terms of reducing oxidative stress, which is beneficial to human health, but also provide protection in food packaging and preservation or used in cosmetic formulations (Marcos *et al*., 2014; Namal Senanayake, 2013; Khalid *et al*., 2018). Plants, especially medicinal herbs, are the best source of natural antioxidants with abundant secondary metabolites, such as phenolic acids and flavonoids. Because they are safe and harmless to humans, the search for natural antioxidants from plants or their extracts has been pay more and more attention (Salem *et al*., 2011; Namal Senanayake, 2013; Feng & Xu, 2014; Costa & Santos, 2017).

The extracts of *Salvia* species exhibit powerful antioxidant capacity (Lu & Fu, 2001; Matkowski *et al*., 2008; Zhang *et al*., 2010; Farhat *et al*., 2013). *S. przewalskii* Maxim (in genus *Salivia*) is a type of traditional medicine plant that is widely distributed in the western part of China (Flora of China Editorial Committee, 1977), and it circulates in the market as a substitute for *S. miltiorrhiza*, which is one of the most important traditional Chinese medicinal plants. *S. przewalskii* Maxim can activate collateral flow, stop pain, promote blood circulation, restore menstrual flow, and ease mental disturbances (Gansu food and drug administration, 2009). This plant also displays anti-HIV (Xu *et al*., 2006; Xu *et al*., 2007), antioxidant (Xue *et al*., 1999; Ozarowski *et al*., 2017), and antibacterial activities. *S. przewalskii* Maxim extract inhibits aldose reductase (Kasimu *et al*., 1998) and treats hepatitis and chronic renal failure (Jiang *et al*., 2013) because of its sharing components, such as tanshinone IIA, salvianolic acid B, and rosmarinic acid.

Many plants, especially medicinal plants, display antioxidant activities due to the abundance of bioactive substances in their tissues. Secondary metabolites, such as flavonoids and phenolic acids, are among the most important bioactive substances regarding antioxidant activities in plants. However, the biosynthesis of these metabolites in plants is influenced by genetics and physical environment (Gorelick & Bernstein, 2014). These metabolites also vary with plant growth and biologically active substance accumulation. Furthermore, the main bioactive components of *S. miltiorrhiza* change at different growth stages (Qin *et al*., 2009; Wang *et al*., 2011). The antioxidant activities of the root and leaf extracts of *S. miltiorrhiza* are well-acknowledged, and specifically prepared *S. miltiorrhiza* leaf extracts have been used as health products in China (Matkowski *et al*., 2008; Zhang *et al*., 2010). In the past two or three

decades, the investigation of *S. przewalskii* has mainly concentrated on the determination of its chemical constituents and active components (Xue *et al*., 2000; Yang *et al*., 2010) and assessment of its quality, pharmacological properties (He *et al*., 2003), and planting technique (Weng *et al*., 2010). Nonetheless, available knowledge is unsatisfactory regarding the variations in the main bioactive composition and antioxidant activities of roots and leaves at different growth stages.

Therefore, this work aims to assess the antioxidant activities of leaf and root extracts from *S. przewalskii* at different growth stages and the eight main bioactive components in root and leaf methanolic extracts. The correlation between these components and antioxidant activity was also analyzed. This study can enhance our understanding of antioxidant activity and provides the necessary basis for further research on antioxidant mechanisms.

Materials and Methods

Materials, reagents and apparatus: *S. przewalskii* seeds were purchased from Shouyang Town, Dingxi City, Gansu Province. The seeds were then sowed on the Maoxian Base (N: 32°09′05.6″, E: 103°35′07.8″; altitude: 2634 ± 10 m) of Sichuan Province. *S. przewalskii* samples were collected at different developmental stages, namely, seeding, squaring, flowering, and harvest stages, as determined by Prof. Yonghong-Zhou of Sichuan Agricultural University. Following the collection and segregation of the samples into underground and aboveground components, the samples were brushed to remove soil, dried under shade, comminuted into powder, and then stored in a desiccator for quantitative determination and antioxidant analysis.

Eight authentic standards were purchased from the National Institute for Food and Drug Evaluation (Beijing, China). We obtained analytical grade formic acid, methanol, ethanol, potassium ferricyanide, ferric trichloride, trichloroacetic acid, and phosphate buffer (pH=6.6) from Chengdu Chemical Reagent Co. Ltd. (Chengdu, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) was sourced from Sigma (St. Louis, MO, USA). We purchased nitro blue tetrazolium (NBT), phenazine methosulfate (PMS), and reduced nicotinamide adenine dinucleotide (NADH) from Sigma Co., Ltd. (Sigma, USA). HPLC-grade methanol and acetonitrile were obtained from Fisher Scientific (Fisher, USA). A Shimadzu CBM-20 Alite HPLC system composed of UV detector and Agilent Eclipse XDB-C18 column (250 mm × 4.6 mm i.d.) was used in this study.

Preparation of extracts: The sample solutions for quantitative determination and antioxidant analysis were prepared as follows. A precisely weighed 0.3 g of sample was transferred into an Erlenmeyer flask containing 50 mL of 70% aqueous methanol and then extracted by an ultrasonic cleaner at an ultrasonic power, temperature, and duration of 240 W, 40°C, 0.5 h, respectively. After cooling to room temperature, the solution was mixed with 70% methanol until the original mass was reached. The solution was then filtered through a 0.45 μm membrane before HPLC analysis.

Preparation of standard substances: Eight standards were accurately weighed and dissolved in HPLC-grade methanol for HPLC analysis. The standard solutions were diluted to various concentrations prior to chromatographic analysis to construct calibration curves ranging from 0.13 mg/g to 7.63 mg/g (protocatechualdehyde), 2.33 mg/g to 256.61 mg/g (rosmarinic acid), 12.51 mg/g to 125.10 mg/g (salvianolic acid B), 0.25 mg/g to 12.24 mg/g (salvianolic acid), 0.64 mg/g to 38.49 mg/g (dihydrotanshinone I), 0.71 mg/g to 53.28 mg/g (tanshinone I), 4.43 mg/g to 44.32 mg/g (cryptotanshinone), and 4.92 mg/g to 73.92 mg/g (tanshinone IIA). All the standards were filtered through a 0.45 μm membrane before HPLC analysis.

Antioxidant activities analysis: The reducing power was determined in accordance with the existing method (Shukla *et al*., 2012) with some modifications. The absorbance of the mixed reaction solution was measured at 710 nm, and a high absorbance of the mixed solution corresponds to a strong reducing power.

DPPH free radical-scavenging activity was determined by the method proposed by Zahin *et al*., (2010) but with some modifications. The absorbance of the mixed solution to the blank was measured at 517 nm using a spectrophotometer. The inhibition percent was calculated using the equation:

$$\text{DPPH radical scavenging rate (\%)} = (A_{control} - A_{sample})/A_{\text{control}} \text{ x } 100$$

where A_{blank} is the absorbance of the control, which contained all reagents except for the test sample, and A_{sample} is the absorbance of the test sample mixed with DPPH radical solution.

Superoxide anion radical-scavenging activity was measured according to the NADH–PMS–NBT method (Masuoka *et al*., 2012) with some modifications. The absorbance of the mixed solution was read by a spectrophotometer at 560 nm. Inhibition percent was computed using the equation:

$$\text{Superoxide radical scavenging rate (\%)} = (1 - A_{sample} / A_{control}) \text{ x } 100$$

where A_{blank} is the absorbance of the control reaction, which contained all reagents except for the test sample, and A_{sample} is the absorbance of the test sample.

Determination of the contents of bioactive components: The bioactive component contents were determined through simultaneous quantitative analysis. The mobile phase included 0.1% formic acid aqueous solution (A) and acetonitrile (B). The linear gradient program was as follows: 0–20 min, from 17% to 49% (B); 20–30 min, from 49% to 51% (B); 30–40 min, from 51% to 60% (B); 40–45 min, from 60% to 68% (B); 45–60 min, from 68% to 74% (B); 60–70 min, from 74% to 17% (B); and 70–73 min, a constant 17% (B). The column temperature, flow rate, and detector wavelength were maintained at 30°C, 0.8 mL/min, and 275 nm, respectively. Approximately 10 μL of the sample was injected, and all determinations were conducted in triplicate.

Results and Discussion

Antioxidant activities: The antioxidant activities of *S. przewalskii* extracts at different stages are shown in Table 1. A high antioxidant activity is represented by a low IC_{50} (EC_{50}) value, and the three tests revealed considerably antioxidant activities in the *S. przewalskii* extracts. The activity levels varied in samples collected at different stages. The reduction capabilities of the extracts weakened with growth. By contrast, the leaf and root extracts obtained at the seeding and squaring stages did not considerably differ. The EC_{50} value of the root extracts collected at the seeding and fruit stages was only 0.19 mg/mL, thereby indicating their remarkable donation of electrons to reactive free radicals. Zhang *et al*., (2010) reported that these electrons are then converted into stable nonreactive species and finally terminated for the free radical chain reaction. The scavenging activity on DPPH radical in both the leaf and root extracts showed a declining trend from the seeding to the harvest stage. The corresponding IC_{50} values ranged from 0.11 ± 0.01 mg/mL to 0.68 ± 0.01 mg/mL and from 0.08 ± 0.01 mg/mL to 0.55 ± 0.03 mg/mL. The IC_{50} value of the underground parts first decreased from the seeding to the fruit stages and then increased until the productive phase with respect to the scavenging activity on superoxide anion radical.

The antioxidant activities of *S. przewalskii* extracts has rarely been examined at different growth stages. This study demonstrated the variation of antioxidant activities with growth, which may be attributed to the difference in bioactive component contents. In the three antioxidant tests, root extracts displayed lower IC_{50} (EC_{50}) values than leaf extracts at every stage. Matkowski *et al*., (2008) reported that the methanol extracts from *S. przewalskii* root exhibited a lower reducing power than the leaf extracts, as indicated by its low EC_{50} value. However, root extracts showed a higher IC_{50} value for DPPH than the leaf extracts given the difference in their bioactive component contents, which may be ascribed to the growth environment. Thus, antioxidant activities depend not only on the content ratio but also on the nature and synergistic interactions of the antioxidants in the mixture compounds (Salem *et al*., 2011).

The methanol extract of *S. miltiorrhiza* leaf showed a lower EC_{50} value for DPPH radical-scavenging activity than that of the *S. przewalskii* leaf but with a higher reducing power, according to Zhang *et al*., (2010). Initially, the *S. miltiorrhiza* leaf is apparently superior to the *S. przewalskii* leaf in terms of antioxidant activities. However, the biomass of *S. przewalskii* leaves is many times higher than that of *S. miltiorrhiza.* Relative to their morphological characteristics, *S. przewalskii* leaves are monocotyledons, mostly basal, and are apparently 8 cm to 23 cm long and 6 cm to 10 cm wide. By contrast, leaves of *S. miltiorrhiza* are dicotyledons, mostly stem leaves, and are approximately 1.5 cm to 8 cm long and 1 cm to 4 cm wide. Therefore, *S. przewalskii* leaves must be exploited.

HPLC method validation: Data accuracy was assessed based on calibration curves, quantitation limit, detection limits, precision, solution stability, repeatability, and analytical method recovery (Table 2). Precision was measured through intra-day variation, and the sample solution was injected six times in a row under the same chromatographic conditions for analysis. The relative standard deviation (RSD) value of retention time and peak areas ranged from 1.5% to 2.7% in the eight standards, thereby indicating that the data were reasonably precise. The intra-day stability of the test solution was measured at 0, 2, 4, 6, 8, 10, 12, and 24 h after preparation. The RSD values of the components ranged from 1.2% to 3.4%, thereby suggesting the stability of the components of the test solution. Method repeatability was evaluated by analyzing the six samples of a single population with a constant preparation procedure. The RSD values of the component contents ranged from 1.4% to 2.1%, thereby indicating that the analytical method was dependable. This result was validated by the standard addition method. Data recovery was calculated as follows:

Recovery (%) = (amount detection-original amount) / amount added × 100.

The RSD value of component recovery ranged from 92.7% to 103.2%. Thus, the accuracy and veracity of the method were verified for the determination of these components.

Table 1. The reducing power and scavenging activity on DPPH radical and superoxide anion radical of *S. przewalskii* extracts at different stage.

Part	Stage	EC_{50} (mg/mL) on reducing power	IC_{50} (mg/mL) on DPPH·	IC_{50} (mg/mL) on O_2^{-}·
Leaf	I[a]	0.30 ± 0.01[b] ^{Ee} [c]	0.11 ± 0.01^{EFe}	0.86 ± 0.03^{De}
	II	0.30 ± 0.02^{Ee}	0.12 ± 0.01^{Ee}	1.13 ± 0.02^{Cc}
	III	0.68 ± 0.02^{Cc}	0.32 ± 0.01^{Cc}	0.70 ± 0.02^{Ef}
	IV	1.92 ± 0.02^{Aa}	0.68 ± 0.01^{Aa}	2.92 ± 0.05^{Aa}
Root	I	0.19 ± 0.02^{Ff}	0.08 ± 0.01^{FGf}	1.07 ± 0.05^{Cd}
	II	0.19 ± 0.02^{Ff}	0.07 ± 0.01^{Gf}	0.49 ± 0.02^{Fg}
	III	0.34 ± 0.02^{Dd}	0.21 ± 0.02^{Dd}	0.55 ± 0.03^{Fg}
	IV	1.61 ± 0.01^{Bb}	0.55 ± 0.03^{Bb}	1.35 ± 0.03^{Bb}

[a] (I) flowering stage; (II) fruit stage; (III) late growth stage; (IV) harvest stage.
[b] Mean ± SD (n=3).
[c] In the same column, different lowercase superscripts mean significant difference at $p<0.05$, values with different capital letter superscripts mean significant difference at $p<0.01$

Table 2. Calibration plot and linear range for the eight compounds.

Compounds	Regression equation[a]	R^{2}[b]	Linear range (μg/mL)	LOD[c] (μg/mL)	LOQ[d] (μg/mL)
Protocatechualdehyde	$y=2.5784e^{-7}x+5.3700e^{-4}$	0.9996	0.13-7.63	0.01	0.03
Rosmarinic acid	$y=7.3510e^{-7}x+3.2951e^{-3}$	0.9999	2.33-256.61	0.19	0.63
Salvianolic acid B	$y=9.5378e^{-7}x+2.3756e^{-3}$	0.9998	12.51-125.10	0.14	0.47
Salvianolic acid A	$y=4.3471e^{-7}x-4.8371e^{-5}$	0.9990	0.25-12.24	0.02	0.07
Dihydrotanshinone I	$y=2.1303e^{-7}x+1.6577e^{-3}$	0.9999	0.64-38.49	0.01	0.02
Tanshinone I	$y=9.9285e^{-8}x-9.8003e^{-3}$	0.9998	0.71-53.28	0.01	0.05
Cryptotanshinone	$y=2.0851e^{-7}x-7.4817e^{-3}$	0.9998	4.43-44.32	0.03	0.11
Tanshinone IIA	$y=1.3143e^{-7}x-4.1112e^{-4}$	0.9999	4.92-73.92	0.03	0.11

[a] In the regression equation $y= ax + b$, y refers to the concentration of the compounds, and x refers to the peak area

[b] R^{2}: the square value of the correlation coefficient of the equation

[c] LOD: the limit of detection

[d] LOQ: limit of quantitation

Table 3. Contents of eight bioactive components in *S. przewalskii* extracts at different stage (mg/mL).

Part	Stage	Protocatechualdehyde	Rosmarinic acid	Salvianolic acid B	Salvianolic acid A	Dihydrotanshinone I	Tanshinone I	Cryptotanshinone	Tanshinone IIA
Leaf	I[(a)]	$0.18 \pm 0.02^{(b)\ Bb\ (c)}$	21.23 ± 0.04^{Cc}	1.16 ± 0.02^{Ff}	0.71 ± 0.01^{Aa}	0.13 ± 0.02^{Ee}	Ud*[(d)]	Ud*	0.02 ± 0.01^{Dd}
	II	0.14 ± 0.01^{Cc}	21.20 ± 0.10^{Cc}	1.05 ± 0.04^{Ff}	0.49 ± 0.02^{Cc}	0.09 ± 0.01^{Ef}	Ud*	Ud*	Ud*
	III	0.12 ± 0.01^{Cc}	14.87 ± 0.13^{Ee}	1.91 ± 0.02^{Ee}	0.60 ± 0.03^{Bb}	0.12 ± 0.02^{Eef}	Ud*	Ud*	Ud*
	IV	0.02 ± 0.01^{Dd}	2.40 ± 0.03^{Ff}	0.52 ± 0.01^{Gg}	0.29 ± 0.02^{Dd}	0.12 ± 0.02^{Eef}	Ud*	Ud*	Ud*
Root	I	0.40 ± 0.01^{Aa}	40.52 ± 0.07^{Aa}	30.32 ± 0.16^{Bb}	0.01 ± 0.01^{Gg}	0.83 ± 0.02^{Dd}	0.34 ± 0.03^{ABb}	0.28 ± 0.01^{Aa}	2.15 ± 0.05^{Aa}
	II	0.14 ± 0.02^{Cc}	31.15 ± 0.06^{Bb}	39.92 ± 0.05^{Aa}	0.03 ± 0.01^{Gg}	1.59 ± 0.03^{Bb}	0.40 ± 0.03^{Aa}	0.26 ± 0.01^{Aa}	2.11 ± 0.09^{Aa}
	III	0.03 ± 0.01^{Dd}	20.75 ± 0.10^{Dd}	12.55 ± 0.03^{Cc}	0.12 ± 0.01^{Ff}	1.16 ± 0.03^{Cc}	0.17 ± 0.02^{Cc}	0.12 ± 0.03^{Bb}	1.25 ± 0.03^{Cc}
	IV	0.03 ± 0.01^{Dd}	1.33 ± 0.03^{Gg}	2.76 ± 0.03^{Dd}	0.23 ± 0.02^{Ee}	1.85 ± 0.02^{Aa}	0.31 ± 0.03^{Bb}	0.09 ± 0.01^{Bc}	1.41 ± 0.03^{Bb}

[(a)] (I) flowering stage; (II) fruit stage; (III) late growth stage; (IV) harvest stage

[(b)] Mean ± SD (n=3).

[(c)] In the same column, different lowercase superscripts mean significant difference at $p<0.05$, values with different capital letter superscripts mean significant difference at $p<0.01$.

[(d)] Ud* showed that content of active components were lower than limit of quantification (LOQ)

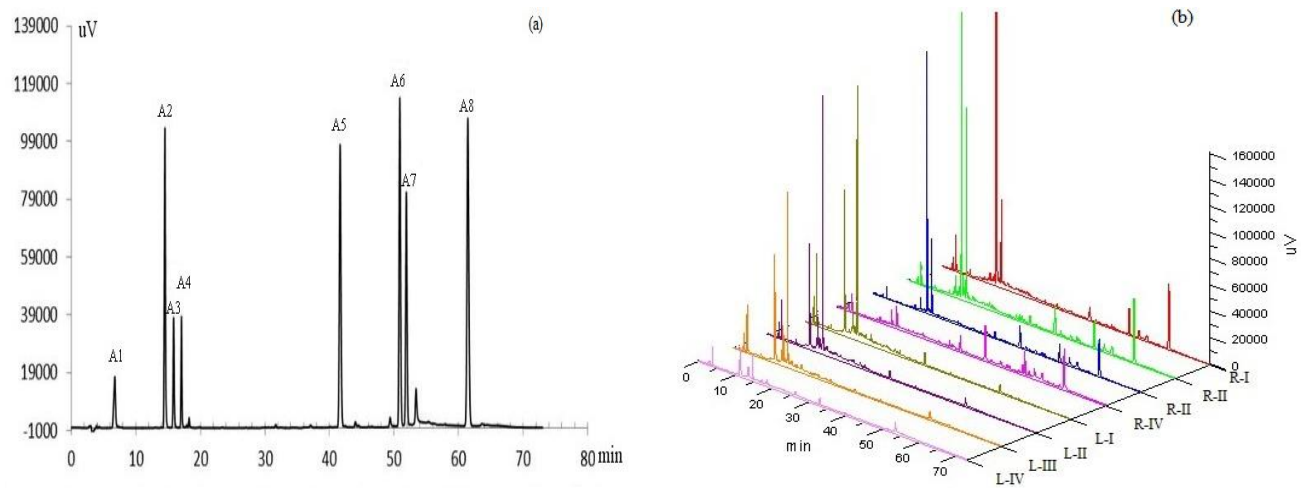

Fig. 1. HPLC chromatograms of (a) standard substances and (b) samples. Peak: (A1) protocatechualdehyde, (A2) rosmarinic acid, (A3) salvianolic acid B, (A4) salvianolic acid A, (A5) dihydrotanshinone I, (A6) tanshinone I, (A7) cryptotanshinone, (A8) tanshinone IIA. (L-I, L-II, L-III, and L-IV represent flowering stage, fruit stage, late growth stage, and harvest stage of leaves, respectively; R-I, R-II, R-III, and R-IV represent flowering stage, fruit stage, late growth stage, and harvest stage of roots, respectively).

Qualitative and quantitative analyses of eight bioactive components: The eight bioactive components of all samples were qualitatively and quantitatively analyzed under optimal chromatographic conditions in triplicate. The chromatograms of the standard substances and of the samples are depicted in Fig. 1. Under optimized conditions, the samples were satisfactorily separated with good resolution. The quantitative data were calculated according to their respective calibration curves (Table 2) and are presented in Table 3. The contents of the eight bioactive components considerably varied at different growth stages.

This study showed that the content of protocatechualdehyde and rosmarinic acid in the leaf and root extracts decreased from flowering to harvesting. Nonetheless, these concentrations were high at the flowering stage of the roots for the leaf and root extracts at 0.40 ± 0.01 and 40.52 ± 0.07 mg/g, respectively. The contents of salvianolic acids B and A dropped from the seeding to the fruit stages and then increased at the late growth stage in the leaves. These concentrations then declined again at the harvest stage. The contents of salvianolic acid B increased from the seeding to the fruit stage and then declined in the roots, whereas salvianolic acid A concentrations increased from the flowering stage to the harvest stage. Nonetheless, the contents of salvianolic acid A at 0.71–0.29 mg/g were higher in the leaves than in the roots at 0.01–0.23 mg/g at every detected stage. The content of salvianolic acid B reached its maximum level at the late growth stage in the roots at 39.92 ± 0.05 mg/g. This concentration was nearly 20 times higher than that in the leaves. Thus, rosmarinic acid and salvianolic acid B were the main components of the *S. przewalskii* leaf extracts. Several leaves are discarded as waste during the harvest of *S. przewalskii* roots to satisfy the continually increasing market demand for this plant. Therefore, the potential of this valuable resource should be evaluated from the waste utilization perspective, because it produces natural phenolic antioxidants when collected at the squaring or late growth stage.

This component content in the root extracts failed to reach its optimum value at the seeding and fruit stages, as with tanshinone I and cryptotanshinone. These components' contents were also insignificant at $p<0.01$ level. Dihydrotanshinone I was the sole lipophilic component detected in the leaf extracts at the considered stages. This component reached its maximum content of 1.85 ± 0.02 mg/mL at the harvest stage of the root extracts and not in the other three stages. By contrast, the trace amounts of tanshinone IIA are insignificant at the $p<0.01$ level in the flowering stage.

S. przewalskii are cultivated in Maoxian County, Sichuan Province, which is located in high-altitude areas with an average elevation of 2500 m. The annual precipitation in this region is 600 mm and is mainly concentrated in from May to October. The annual mean temperature is 6°C; this temperature drops to 2.8°C to 8°C in January and increases to 22.5°C in July (Tang & Tian, 2013). Therefore, *S. przewalskii* mainly grows from May to October because of the rainfall distribution and temperature. This growth period is shorter than that of plants cultivated in low-altitude areas. *S. przewalskii* first enters its vegetative period from June to July. At this time, the main metabolites transiently accumulate. This plant then enters the late growth stage from July to August or September. The contents of the main secondary metabolite decrease due to numerous metabolites used in reproductive growth. As the plant enters the second vegetative period, the secondary metabolites accumulate abundantly and are then transferred to the roots from the leaves. The roots then enter the swelling stage, in which the weight increases rapidly. The difference between the expansion rate of the roots and the accumulation rate of effective components is the reason for the differences in the main bioactive components' contents.

Table 4. Correlation analysis between with eight active compounds and antioxidant activities.

	A1[a]	A2	A3	A4	A5	A6	A7	A8
B1[b]	0.685	0.944**	0.744*	-0.241	0.125	0.418	0.641	0.505
B2	0.799*[c]	0.952**	0.747*	-0.211	0.093	0.437	0.663	0.520
B3	0.278	0.731*	0.535	-0.168	0.097	0.200	0.396	0.294

[a] A1: Protocatechualdehyde, A2: Rosmarinic acid, A3: Salvianolic acid B, A4: Salvianolic acid A, A5: Dihydrotanshinone I, A6: Tanshinone I, A7: Cryptotanshinone, A8: Tanshinone IIA.

[b] B1: Reducing power, B2: DPPH radical scavenging activity, B3: Superoxide anion radical scavenging activity

[c] * = $p<0.05$, ** = $p<0.01$

Relationship between the antioxidant activities and bioactive components: Table 4 exhibits the analysis results of the correlation between antioxidant activities and the contents of the bioactive components. Reducing power was significantly correlated with salvianolic acid B and rosmarinic acid at $p<0.05$ and $p<0.01$ levels, respectively. Meanwhile, DPPH radical scavenging activity was significantly correlated with protocatechualdehyde ($p<0.05$), salvianolic acid B ($p<0.05$), and rosmarinic acid ($p<0.01$). Superoxide anion radical-scavenging activity was significantly correlated with rosmarinic acid at the $p<0.05$ level. However, the content of salvianolic acid A was negatively correlated with reducing power and DPPH radical, and superoxide anion radical-scavenging activity. The correlation with lipophilic components was unremarkable. The phenolic compounds in plants are highly effective free radical scavengers and antioxidants (Farhat *et al*., 2013; Maria *et al*., 2014; Lim *et al*., 2019). The results of the present study were consistent with those of previous studies, in which the contents and antioxidant activities of leaf and root extracts were collected at different stages. Zhang *et al*., (2010) reported that salvianolic acid B and rosmarinic acid are major contributors to the antioxidant activities of *S. miltiorrhiza*. Matkowski *et al*., (2008) indicated that salvianolic acid B predominantly contributes to the antioxidant activities in both root and leaf extracts, followed by rosmarinic, ferulic, and caffeic acid. Nonetheless, the present results also suggested that protocatechualdehyde was significantly correlated with DPPH radical scavenging activity at $p<0.05$ level. Furthermore, rosmarinic acid was significantly correlated with reducing power and DPPH radical-and superoxide anion radical-scavenging activities at $p<0.01$ and $p<0.05$ levels, respectively. This finding may denote the main difference between *S. przewalskii* and *S. miltiorrhiza*.

Conclusion

The antioxidant activities and contents of eight bioactive components in grass roots and leaf extracts at different growth stages were first conducted in the study. This study clarified the changes in their antioxidant activities and the contents of the eight bioactive components in the extracts from the flowering stage to the harvest stage and the relationship between antioxidant activities and the main bioactivity components in *S. przewalskii* extracts. The antioxidant activities and the contents of the bioactive components of the leaf and root extracts varied considerably when collected in different growth periods. Excluding salvianolic acid A, the contents of the hydrophilic components, i.e., protocatechualdehyde, rosmarinic acid, and salvianolic acid B, were considerably correlated with the antioxidant activities of the extracts, including reducing power and DPPH radical- and superoxide anion radical-scavenging activities, especially rosmarinic acid. Thus, *S. przewalskii* leaves can be used as remarkably powerful herbal antioxidants when collected after flowering stage because of their high hydrophilic component contents. Therefore, further research on antioxidative mechanism and how to develop and utilize the leaves is still necessary to fully improve the commercial value of *S. przewalskii*.

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