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Analysis and comparison of the levels of bioactive components in roots of *Salvia miltiorrhiza Bunge* from different germplasms by high-performance liquid chromatography

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Abstract

The objectives of this study were to analyze the bioactive components (BC), including both hydrophilic components (HC) (i.e., danshensu (DSS), protocatechuic aldehyde (PTAL) and salvianolic acid B (SAB) and lipophilic components (LC) (i.e., cryptotanshinone (CTS) and tanshinone IIA (TS IIA), in the roots of *Salvia miltiorrhiza* from different germplasms and with different root colors, diameters, and cortex/xylem ratios. Twelve *S. miltiorrhiza* germplasms were collected from 12 areas in 6 provinces of China. All 12 germplasm lines were planted in a field at the Institute of Medicinal Plant Development in 2009 and 2010. The BC in roots of the tested *S. miltiorrhiza* materials were measured simultaneously using high-performance liquid chromatography (HPLC). Of the 11 germplasms tested, DS007 from *Henan Yuzhou* had the largest amounts of HC and LC (i.e., DSS, PTAL, SAB, CTS, and TSIIA). In all germplasms investigated, the DSS, SAB, CTS and TSIIA contents were higher in the cortex (including the phloem and cork layers) than in the xylem. The CTS and TSIIA were more abundant in the roots with smaller diameters. The CTS (0.023%, w/w), TSIIA (0.054%, w/w) and SAB (4.76%, w/w) levels were lowest in the lutou (basal or residual part of stem), while the DSS (0.061%, w/w) and PTAL (0.005%, w/w) contents were highest in this portion. The amounts of both HC and LC were higher in more deeply colored (brownish-red) root powders.

Keywords: *Salvia miltiorrhiza Bunge*; comparison; HPLC; hydrophilic component; lipophilic component.
Abbreviations: HPLC-high-performance liquid chromatography; BC-bioactive components; HC-hydrophilic components; LC-lipophilic components; DSS-danshensu; PTAL-protocatechuic aldehyde; SAB-salvianolic acid B; CTS-cryptotanshinone; TSIIA-tanshinone IIA.

Introduction

Red sage root (local term, "Danshen"), the root and rhizome of *Salvia miltiorrhiza Bunge* (Fam. *Labiatae*), is commonly used in traditional Chinese medicine (TCM) to improve body function, e.g., to promote circulation and improve blood flow (Li et al., 2008). It has been widely used for the treatment of cardiovascular disease, cerebrovascular disease, coronary heart disease, chronic renal failure, atherosclerosis, myocardial infarction, angina pectoris, myocardial ischemia, dysmenorrhea, neurasthenic insomnia, and liver fibrosis and cirrhosis (Wasser et al., 1998; Liu et al., 2000; Chae et al., 2004; Ling et al., 2005; Matkowski et al., 2008; Dong et al., 2010). Chemical and pharmacological investigations have identified 2 groups of bioactive components (BC) in *S. miltiorrhiza*: phenolic acids, including hydrophilic compounds such as salvianolic acid A, salvianolic acid B (SAB), rosmarinic acid, lithospermic acid, caffeic acid, danshensu (DSS), and protocatechuic aldehyde (PTAL), and tanshinones, which are lipophilic and include tanshinone IIA (TS IIA), tanshinone I, tanshinone IIB, and cryptotanshinone (CTS) (Lu et al., 2002; Zhang et al., 2003; Du et al., 2004; Seo et al., 2005). TSIIA and SAB are both considered by the Chinese Pharmacopoeia to be marker compounds for *S. miltiorrhiza* quality control (TPC, 2010). Furthermore, DSS and PTAL have been identified as the main water-soluble constituents (Li et al., 2008; Luo et al., 2008). TSIIA is reported to be distributed mainly in the root phloem and cork and less in the xylem, while SAB is present mainly in the root xylem and phloem and less in the cork (Qu et al., 2005; Zeng et al., 2006). The level of TSIIA is higher in the fine and fibrous roots of *S. miltiorrhiza* (Hu and Wang, 2005) and is highest in the smallest-diameter roots (Liu and Li, 2007). Yang et al. (2007) investigated the relationship between the root skin color and BC content of *S. miltiorrhiza* and confirmed that the best-quality *S. miltiorrhiza* roots were those with a brownish-red skin color. The skin color is similar among most *S. miltiorrhiza* roots, while the powder colors may be quite different. However, little is known about the relationship between the color of *S. miltiorrhiza* root powder and its active component content. Moreover, *S. miltiorrhiza* is produced in different areas of China and comes from various germplasms. The BC content is reported to vary among samples of different origins and *Salvia* varieties (Jian et al., 1989; He et al., 2010). It is therefore necessary to study the variations in the contents of the main BC in the roots of *S. miltiorrhiza* from different germplasms. The morphological characteristics of *S. miltiorrhiza* roots are

closely related both to their germplasms and to environmental factors (Li et al., 2008). Because there are many *S. miltiorrhiza* germplasms in different regions of China, the root characteristics, such as the color, diameter, and cortex/xylem ratio, are varied. In short, one would expect significant differences in the amounts of BC in *S. miltiorrhiza* roots from different germplasms with different root colors, diameters, and xylem/cortex ratios. Therefore, the evaluation of *S. miltiorrhiza* with respect to the above factors would be essential for the quality control of *S. miltiorrhiza* germplasm resources. In this experiment, germplasms with stable active components and consistent morphological root characteristics (e.g., brownish-red color, smaller diameter, and larger cortex/xylem ratio) were selected for analysis to avoid the potential effects of inconsistent and unstable materials and components. This study was designed to analyze the contents of 5 BC (DSS, PTAL, SAB, CTS, and TSIIA) in the roots of *S. miltiorrhiza* from different germplasms and compare the BC contents of *S. miltiorrhiza* roots with different xylem/cortex ratios, root diameters, and root (skin and power) colors. These data should provide a basis for a selection standard for breeding germplasms for desirable characteristics.

Results and discussion

Amounts of the bioactive components in different S. miltiorrhiza germplasms

The amounts of danshensu (DSS), protocatechuic aldehyde (PTAL), salvianolic acid B (SAB), cryptotanshinone (CTS), and tanshinone IIA (TSIIA) in the roots of 11 *S. miltiorrhiza* germplasms from different areas of China (Fig. 1; Table 1) are shown in Table 2. Similarly to Jin et al. (2004), we found that the levels of these major bioactive components (BC) in *S. miltiorrhiza* roots differed significantly among the 11 germplasms. The SAB and total hydrophilic contents were higher in DS003 (115.1 and 116.1 mg g^{-1}, dry weight (DW), respectively), DS004 (95.3 and 96.2 mg g^{-1}, DW, respectively), and DS007 (94.3 and 95.2 mgg^{-1}, DW, respectively) than in the other *S. miltiorrhiza* germplasms, while the CTS, TSIIA, and total lipophilic contents were highest in DS007 (0.82, 1.88, and 2.70 mg g^{-1}, DW, respectively) from *Henan Yuzhou*. Together, these results suggested that DS007 was the best of these germplasms, and indeed, Liu et al. (2004) had previously reported that the amounts of hydrophilic and lipophilic components (HC and LC) were higher in the roots of *S. miltiorrhiza* from *Henan Yuzhou* than in those of other germplasms investigated. Data variance analysis showed that the amounts of DSS, SAB, and TSIIA differed significantly among the *S. miltiorrhiza* germplasms. Although the SAB contents in the roots of *S. miltiorrhiza* were much higher than the 3% required by the Chinese Pharmacopoeia, most of the investigated germplasms did not meet the TS level requirement of the Chinese Pharmacopoeia (0.2%) (TPC, 2010). The amounts of DSS, PTAL, and CTS were very low, and the levels of PTAL and CTS were the lowest among the 5 BC and in some cases were undetectable in the *S. miltiorrhiza* roots. This result agreed well with the findings of Ni et al. (1998) and Wang et al. (2005) but differed from those reported by Jin et al. (2004). The *S. miltiorrhiza* materials used in previous studies were very complex and were collected from different regions after growing under varying cultivation and environmental conditions; therefore, the differences in the levels of active compounds could have been related to both genetic and environmental factors. However, the samples of *S. miltiorrhiza* used in this study were collected from the same experimental field and had been grown in the same climatic, soil, and cultivation conditions, so environmental factors had little or no influence on the BC of the *S. miltiorrhiza* roots analyzed here. One possible explanation is that the different results observed were due mainly to genetic or germplasm differences and evaluating other reasons for this result would require further analysis and verification.

Comparison of the levels of bioactive components in different parts of S. miltiorrhiza roots

Four *S. miltiorrhiza* germplasms (DS004, DS012A, DS010, and DS012B) were chosen as the experimental materials. The xylem/cortex ratios of the roots of these *S. miltiorrhiza* germplasms were similar for some varieties and distinct for others, and 5 plants with consistent roots were chosen for each germplasm for repeated analysis (Table 3). Table 4 shows that the TSIIA and CTS levels were significantly higher in the cortex (which included the phloem and cork layers in this study) than in the xylem across all 4 germplasms. The DSS level was also significantly higher in the cortex than in the xylem for all 4 *S. miltiorrhiza* germplasms. While the SAB level too was higher in the cortex than in the xylem for all germplasms, the difference was only significant for DS010. The PTAL level was much higher in the cortex than in the xylem in DS010, did not differ between the 2 parts in DS012B, and was not detectable in DS004 and DS012A, suggesting that the PTAL level in *S. miltiorrhiza* roots is germplasm-dependent. Moreover, these results indicate that the xylem/cortex diameter ratio has little effect on the contents of these 5 BC in *S. miltiorrhiza*. Our results for the patterns of distribution of BC in *S. miltiorrhiza* roots were similar to those reported by Zeng et al. (2006) and Qu et al. (2005) for TSIIA and SAB and constitute the first such report for DSS, PTAL, and CTS.

Comparison of the levels of bioactive component in S. miltiorrhiza roots of different diameters

Two *S. miltiorrhiza* germplasms (DS012B and DS010) were selected as the experimental materials, and 5 plants from each germplasm were used for repeated analysis. The roots and rhizomes of *S. miltiorrhiza* were divided into 5 grades: lutou (basal or residual part of stem), I (root diameter: 6–8 mm), II (4–6 mm), III (2–4 mm), and IV (<2 mm), as shown in Table 3. In both germplasms investigated, the levels of lipophilic components (LC) (CTS and TSIIA) were highest (0.127 and 0.328%, w/w) in grade IV roots, followed by grade III, II, and I roots, and were lowest (0.023 and 0.054%, w/w) in the lutou, although the difference between grades II and I was not significant in any case. The relative hydrophilic component (HC) (DDS, PTAL, and SAB) contents of the 4 grades of *S. miltiorrhiza* roots were, in descending order, I > II > III > IV, and the levels of DDS and PTAL did not differ significantly among the 4 grades. For both germplasms, the *S. miltiorrhiza* lutou contained the highest amounts of DDS (0.061%, w/w) and PTAL (0.005%, w/w) and the lowest amount of SAB (4.76%, w/w). These results indicate that the TSIIA content of *S. miltiorrhiza* roots increases as the diameter decreases, which is consistent with previous reports (Hu and Wang, 2005; Liu and Li, 2007). The effect of the root diameter on the levels of the bioactive components (BC) other than TSIIA has not previously been reported. Our results showed that the CTS and TSIIA contents increased as the *S. miltiorrhiza* root diameter decreased, while the reverse was true for the HC content, as shown in Table 5. TSIIA,

Table 1. Salvia miltiorrhiza samples collected from different areas of China and used in the experiments.

Sample Number	Germplasm origin	Important characteristics
DS001[1]	*Shandong*	Violet flower
DS002	*Shandong*	White flower
DS003	*Jiangsu* (uncultivated)	Violet flower
DS004	*Henan* (uncultivated)	Violet flower
DS005	*Henan* (cultivated)	Violet flower
DS006	*Shaanxi Shangluo*	Violet flower
DS007	*Henan Yuzhou*	Violet flower
DS009A	*Beijing Pinggu* (uncultivated)	Violet flower
DS009B	*Beijing Pinggu* (cultivated)	Violet flower
DS010	*Beijing Pinggu Xilijin*	Violet flower
DS012A	*Hebei* (uncultivated)	Violet flower
DS012B	*Hebei* (cultivated)	Violet flower

[1]Danshen (DS) + Gemplasm Number (001) = DS001

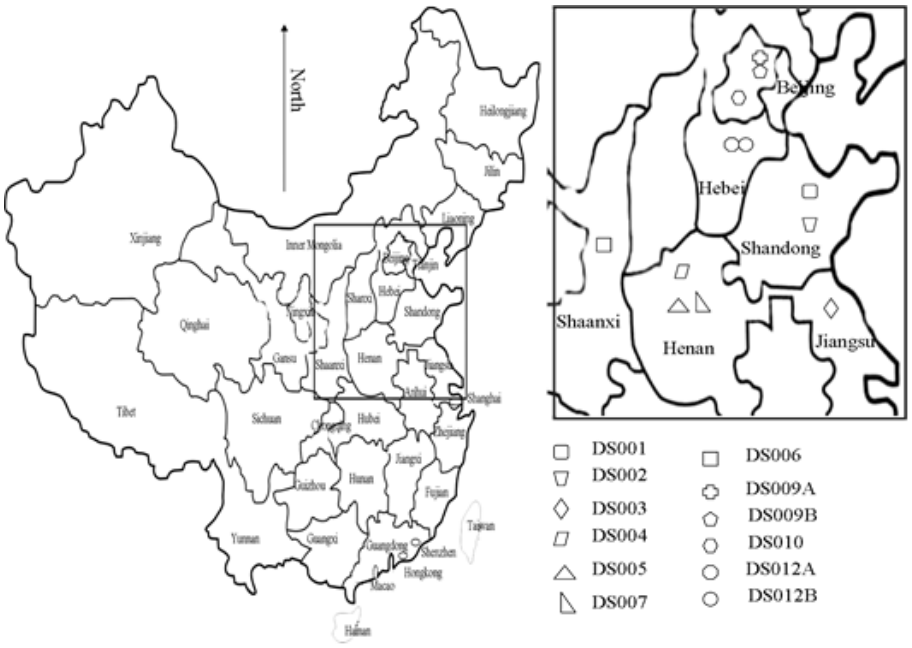

Fig 1. A simple map of China showing the regions in which specimens were collected in 2009–2010.

CTS, and other LC are reported to be synthesized and accumulated mainly by peridermal cells and DSS, PTAL, and SAB mainly by xylem and phloem parenchymal cells (Hu and Wang, 2005). Therefore, it has been thought that the smaller the *S. miltiorrhiza* root diameter, the greater the proportion of cortex and therefore the higher the LC content, while the larger-diameter roots have a greater proportion of xylem and consequently higher levels of HC. The levels of DDS and PTAL were highest and the SAB content, in contrast, was lowest in the *S. miltiorrhiza* lutou. One possible explanation is that the lutou contains the short and small rhizome at the top of the taproot, which has some transverse stripes and probably differs from the taproot in structure and other features. Further study of the relationship between BC content and root structure is warranted. Taken together, these results suggest that discarding the lutou and fine roots of *S. miltiorrhiza* during the initial medicine acquisition and production is not desirable and that pharmaceutical companies should be open to using these parts of the *S. miltiorrhiza* germplasms studied according to their needs.

Comparison of the levels of bioactive components of different-colored S. miltiorrhiza roots

The color of *S. miltiorrhiza* root epidermis is usually brownish-red or red but varies somewhat among the different *S. miltiorrhiza* species. Yang (2007) reported that the roots with a deeper brownish-red color contained higher amounts of the bioactive components (BC). However, we observed that the skin color often did not differ between roots that produced quite different-colored powders (Table 3). Two different-colored adventitious roots from the same plant (DS006) were selected as the experimental materials. The root powder color was determined using a colorimetric card. As shown in Table 6, different-colored *S. miltiorrhiza* roots differed distinctly in their contents of HC (DSS, PTAL, and SAB) and LC (CTS and TSIIA). The roots whose colors were recorded as 7514C, 7515C, 4665C, and 4655C were brownish-red, and the root designated 7514C showed both the deepest color and the highest levels of HC, as well as high levels of LC. The roots whose colors were recorded as

Table 2. Amounts (mg g^{-1} DW) of DSS, PTAL, SA B, CTS, and TSIIA in roots from 11 *S. miltiorrhiza* germplasms**.**

Sample	DSS[3]	PTAL	SAB	Total hydrophilic content	CTS	TSIIA	Total lipophilic content
DS001	0.69 ± 0.02b[1]	nd[2]	89.5 ± 2.69bc	90.2 ± 2.71bc	nd	0.26 ± 0.01ef	0.26 ± 0.01ef
DS002	0.64 ± 0.02b	0.01 ± 0.00a	84.2 ± 2.53cd	84.9 ± 2.55cd	0.18 ± 0.01a	0.79 ± 0.02c	0.97 ± 0.03c
DS003	0.95 ± 0.03a	0.07 ± 0.00a	115.1 ± 3.45a	116.1 ± 3.48a	0.74 ± 0.02b	0.73 ± 0.02cd	1.47 ± 0.04cd
DS004	0.89 ± 0.03a	0.01 ± 0.00a	95.3 ± 2.86b	96.2 ± 2.89b	nd	0.55 ± 0.02cde	0.55 ± 0.02cde
DS005	0.49 ± 0.01d	nd	59.1 ± 1.77f	59.6 ± 1.79f	nd	0.18 ± 0.01f	0.18 ± 0.01f
DS006	0.44 ± 0.01d	0.02 ± 0.00a	55.1 ± 1.65f	55.6 ± 1.67f	nd	0.38 ± 0.01def	0.38 ± 0.01def
DS007	0.89 ± 0.03a	0.02 ± 0.00a	94.3 ± 2.83b	95.2 ± 2.86b	0.82 ± 0.02b	1.88 ± 0.06a	2.70 ± 0.08a
DS009A	0.52 ± 0.02cd	0.01 ± 0.00a	69.7 ± 2.09e	70.2 ± 2.11e	0.69 ± 0.02b	0.51 ± 0.02cdef	1.20 ± 0.04cdef
DS009B	0.61 ± 0.02bc	0.01 ± 0.00a	79.6 ± 2.39d	80.2 ± 2.41d	0.25 ± 0.01a	0.65 ± 0.02cd	0.90 ± 0.03cd
DS010	0.44 ± 0.01d	0.02 ± 0.00a	60.5 ± 1.82f	61.0 ± 1.83f	0.31 ± 0.01a	1.20 ± 0.04b	1.51 ± 0.05b
DS012A	0.63 ± 0.02bc	0.01 ± 0.00a	81.7 ± 2.45cd	82.3 ± 2.47cd	0.12 ± 0.00a	0.59 ± 0.02cde	0.71 ± 0.02cde

[1]Different letters (LSD) indicate significant differences ($p < 0.05$). [2]not detectable. [3]mg g^{-1}, dry weight, (DW), mean (the average values of the 2009 and 2010 samples) ± standard error, ($n = 5$). DSS = danshensu. PTAL = protocatechuic aldehyde. SAB = salvianolic acid B. CTS = cryptotanshinone. TSIIA = tanshinone IIA.

Table 3. Root morphological characteristics of experimental samples of *S. miltiorrhiza*.

Sample	xylem /cortex	Root grade (range of diameter)	Root skin color	Root powder color
DS006-1	—[1]	—	1807C[2]	7514C
DS006-1	—	—	174C	4665C
DS006-2	—	—	1807C	7515C
DS006-2	—	—	174C	7502C
DS006-3	—	—	1807C	4655C
DS006-3	—	—	174C	482C
DS004	0.546	—	—	—
DS012A	0.990	—	—	—
DS010	0.842	Lutou, I (6–8 mm), II (4–6 mm), III (2–4 mm), IV (<2 mm)	—	—
DS012B	0.948	Lutou, I (6–8 mm), II (4–6 mm), III (2–4 mm), IV (<2 mm)	—	—

Lutou = base of root or residual part of the stem. Cortex = including phloem and cork layers. [1] not determined. [2] "C" or "U" was the unit of the color using the C/U special color card, with "C" indicating a bright color and "U" a non-bright color.

7502C and 482C were gray-white and had the lowest levels of BC in the germplams tested. All of the above results suggest that the HC and LC contents are higher in brownish-red powders than in gray or white powders and that the depth of the color correlates positively with the levels of the BC. Therefore, as the skin color does not accurately reflect the levels of BC, the *S. miltiorrhiza* root powder color should be analyzed. Analysis of the BC in *S. miltiorrhiza* samples from 11 germplasms showed that DS007 from *Henan Yuzhou* had high levels of both HC and LC (DSS, PTAL, SAB, CTS, and TSIIA). The levels of DSS, SAB, TIIA, and CTS were higher in the cortex than in the xylem in the 4 germplasms examined. The levels of LC (CTS and TSIIA) were higher in smaller-diameter roots, in contrast to the hydrophilic component (DSS, PTAL, and SAB) content, which increased as the diameter increased. The levels of LC were the lowest in the lutou, while the levels of DSS and PTAL, but not SAB, were highest in that segment. The depth of the color of the root powder (brownish-red) correlated positively with the HC and LC contents.

Material and methods

Plant materials

Specimens of 12 *S. miltiorrhiza* germplasms (No. 001, 002, 003, 004, 005, 006, 007, 009A, 009B, 010, 012A, and 012B, designated in the study as DS001, DS002, DS003, DS004, DS005, DS006, DS007, DS009A, DS009B, DS010, DS012A, and DS012B, where DS is an abbreviation for Danshen, the local term for the plant in China) were collected from 12 areas in 6 provinces in China (Fig. 1; Table 1). The 12 *S. miltiorrhiza* germplasms were authenticated by Professor Jian-he Wei of the Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences (CCAMS) and Peking Union Medical College. Root segments of all 12 germplasm lines were sprouted in an artificial climate chamber and then planted in a field at IMPLAD, CAMS, Beijing, China on May 5, 2009 and May 2, 2010, respectively, using conventional commercial cultivation methods. All germplasm lines were harvested in November 2009 and November 2010. Roots were oven-dried to a constant weight at 60°C, weighed, and then ground to pass through a 0.3-mm sieve. Eleven *S. miltiorrhiza* germplasms (DS001, DS002, DS003, DS004, DS005, DS006, DS007, DS009A, DS009B, DS010, and DS012A) from different areas in China were chosen for comparison of the levels of bioactive components (BC) in their roots. Four *S. miltiorrhiza* germplasms (DS004, DS010, DS012A, and DS012B) with significantly different xylem-to-cortex ratios were selected for separation into the cortex (including cork and phloem) and xylem. Two of these *S. miltiorrhiza* roots (DS012B and DS010) were divided into the lutou (basal part or residual stem portion) and root grades I (root diameter: 6–8 mm), II (root diameter: 4–6 mm), III

Table 4. Comparison of the levels of 5 active components in the cortex and xylem of *S. miltiorrhiza.*

Sample	DSS[3]		SAB		PTAL		CTS	TSIIA		
	Xylem	Cortex	Xylem	Cortex	Xylem	Cortex	Xylem	Cortex	Xylem	Cortex
DS004	0.031 ± 0.00aA[1]	0.068 ± 0.00bB	3.26 ± 0.10aA	3.72 ± 0.11aA	nd[2]	nd	0.002 ± 0.00bB	0.010 ± 0.00aA	0.010 ± 0.00bB	0.062 ± 0.00aA
DS010	0.025 ± 0.00bB	0.045 ± 0.00aA	5.45 ± 0.16bB	8.41 ± 0.25aA	0.005 ± 0.00bB	0.021 ± 0.00aA	0.009 ± 0.00bB	0.095 ± 0.00aA	0.022 ± 0.00bB	0.250 ± 0.01aA
DS012A	0.036 ± 0.00aA	0.059 ± 0.00bB	5.18 ± 0.16aA	6.06 ± 0.18aA	nd	nd	0.006 ± 0.00bB	0.031 ± 0.00aA	0.012 ± 0.00bB	0.066 ± 0.00aA

[1]different letters (LSD) indicate significant differences between the cortex and xylem of a sample, lowercase letters indicate $p < 0.05$ and uppercase letters $p < 0.01$. [2]not detectable. [3]% (w/w), mean (the average values of the 2009 and 2010 samples) ± standard error, ($n = 5$). DSS = danshensu. PTAL = protocatechuic aldehyde. SAB = salvianolic acid B. CTS = cryptotanshinone. TSIIA = tanshinone IIA.

Table 5. Comparison of the levels of 5 active components in roots of *S. miltiorrhiza* with different diameters

Sample	C / R	Lutou	I	II	III	IV
DS012B	DSS[2]	0.052 ± 0.00aA[1]	0.052 ± 0.00aA	0.047 ± 0.00abA	0.046 ± 0.00abA	0.042 ± 0.00bA
	PTAL	0.005 ± 0.00aA	0.003 ± 0.00bAB	0.003 ± 0.00bAB	0.002 ± 0.00bB	0.002 ± 0.00bB
	SAB	4.76 ± 0.14abcAB	7.50 ± 0.23aA	6.58 ± 0.22abAB	6.02 ± 0.18bcAB	5.09 ± 0.16cB
	CTS	0.028 ± 0.00abAB	0.044 ± 0.00bB	0.064 ± 0.00bAB	0.089 ± 0.00bAB	0.127 ± 0.00bA
	TSIIA	0.099 ± 0.00cC	0.146 ± 0.00bcBC	0.176 ± 0.01bcBC	0.225 ± 0.01bAB	0.328 ± 0.01aA
DS010	DSS	0.061 ± 0.00aA	0.044 ± 0.00bB	0.044 ± 0.00bB	0.044 ± 0.00bB	0.045 ± 0.00bB
	PTAL	0.005 ± 0.00aA	0.002 ± 0.00bB	0.002 ± 0.00bB	0.001 ± 0.00bB	0.002 ± 0.00bB
	SAB	4.76 ± 0.15bC	6.74 ± 0.20aA	6.36 ± 0.19aAB	6.10 ± 0.18aAB	5.14 ± 0.16bBC
	CTS	0.023 ± 0.00cC	0.073 ± 0.00bB	0.074 ± 0.00bB	0.104 ± 0.00aAB	0.124 ± 0.00aA
	TSIIA	0.054 ± 0.00cC	0.172 ± 0.01bB	0.169 ± 0.01bB	0.197 ± 0.01bB	0.266 ± 0.01aA

[1]different letters (LSD) indicate significant differences among the different diameters, lowercase letters indicate $p < 0.05$ and uppercase letters $p < 0.01$. [2]% (w/w), mean (the average values of the 2009 and 2010 samples) ± standard error, ($n = 5$). C = component. R = root grade. Lutou = base of root or residual part of the stem. I = root 6–8 mm in diameter. II = root 4–6 mm in diameter. III = root 2–4 mm in diameter. IV = root <2 mm in diameter.

Table 6. Comparison of the levels of 5 active components in different-colored roots of *S. miltiorrhiza*

Sample	Root color	powder	DSS[3]	PTAL	SAB	Total hydrophilic content	CTS	TSIIA	Total lipophilic content
DS006-1	7514C		0.086 ± 0.00aA[1]	0.002 ± 0.00aA	10.07 ± 0.30aA	10.16 ± 0.31aA	0.059 ± 0.00bB	0.170 ± 0.01bB	0.201 ± 0.01bB
DS006-1	4665C		0.077 ± 0.00aA	0.002 ± 0.00aA	9.03 ± 0.27bB	9.11 ± 0.28bB	0.042 ± 0.00aA	0.105 ± 0.00dD	0.146 ± 0.00cC
DS006-2	7515C		0.048 ± 0.00dC	nd	7.78 ± 0.22cC	7.82 ± 0.23cC	0.040 ± 0.00aA	0.197 ± 0.01aA	0.237 ± 0.01aA
DS006-2	7502C		0.034 ± 0.00eD	nd[2]	5.34 ± 0.17eE	5.37 ± 0.16eE	0.008 ± 0.00dD	0.015 ± 0.00fE	0.023 ± 0.00eE
DS006-3	4655C		0.062 ± 0.00bB	0.003 ± 0.00bB	7.79 ± 0.23cC	7.85 ± 0.24cC	0.015 ± 0.00cC	0.114 ± 0.00cC	0.130 ± 0.00dD
DS006-3	482C		0.052 ± 0.00cC	0.003 ± 0.00bB	7.17 ± 0.22dD	7.23 ± 0.22dD	0.002 ± 0.00eE	0.017 ± 0.00eE	0.019 ± 0.00fE

[1]different letters (LSD) indicate significant differences among the different-colored roots, lowercase letters indicate $p < 0.05$ and uppercase letters $p < 0.01$. [2]not detectable. [3]% (w/w), mean (the average values of the 2009 and 2010 samples) ± standard error, ($n = 5$). DSS = danshensu. PTAL = protocatechuic aldehyde. SAB = salvianolic acid B. CTS = cryptotanshinone. TSIIA = tanshinone IIA.

(root diameter: 2–4 mm) and IV (root diameter: <2 mm) according to the parts and diameters of the roots. A germplasm (DS006) that produced different-colored roots from the same plant was chosen to investigate the root skin and powder colors using a colorimetric card (GP 1201, C/U, PANTONE, USA) (Table 3). *S. miltiorrhiza* was propagated clonally, with 30 plants from each germplasm grown in the experimental field. At harvest, each material for each test was collected from 5 plants from each germplasm for analysis.

Chemicals and standards

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (NJ, USA). Deionized water was purified using the Milli-Q system (Millipore, Bedford, MA, USA). Analytical-grade phosphoric acid and ethanol were obtained from Beijing Beihua Fine Chemicals Co., Ltd. (Beijing, China). Authentic standards of danshensu (DSS), protocatechuic aldehyde (PTAL), salvianolic acid B (SAB), cryptotanshinone (CTS), and tanshinone IIA (TSIIA) were purchased from the National Institute for Control of Biological and Pharmaceutical Products (Beijing, China).

Apparatus and analytical conditions

A Waters 1525 HPLC system (Waters Technologies, Milford, MA, USA) comprising a binary solvent delivery system, an on-line degasser, a Waters 717plus autosampler, a column temperature controller, and a Waters 2457 dual γ absorbance detector coupled with an analytical workstation was used. The column was a Waters Symmetry C18 reverse-phase column (5 μm, 250 mm × 4.6 mm). The sample injection volume was 10 μl. The chromatographic conditions for hydrophilic and lipophilic components (HC and LC) were as follows: the detection wavelength was set at 281 nm, the flow rate was 1.0 ml min^{-1}, and the column temperature was maintained at 30°C. A gradient elution with solvents A (acetonitrile) and B (0.05% aqueous phosphoric acid, v/v) was used as follows: 0–10 min, 5–14% A (v/v); 10–12 min, 14–17% A (v/v); 12–16 min, 17–19% A (v/v); 16–20 min, 19–25% A (v/v); 20–35 min, 25% A (v/v); 35–45 min, 25–75% A (v/v); 45–55 min, 75–90% A (v/v); 55–60 min, 90–5% A (v/v); 60–70 min, 5% A (v/v).

Preparation of sample solutions

Each individual sample (0.500 g) was accurately weighed, placed in a 150-ml round-bottom flask, and suspended in 50 ml of 70% methanol. The flask was connected to a condensation tube, placed in a water bath, and extracted under reflux at 75 ± 1°C for 1 h. The mixture was cooled to room temperature, the methanol lost in refluxing was restored, and the extract was centrifuged at 12, 000 rpm at 25°C for 5 min and filtered through a membrane (0.45 μm). A 10-μl volume of the filtrate was injected into the HPLC.

Data transformation and data analysis

Data transformation was performed using MS Excel. Treatment effects were determined by 1-way analysis of variance (ANOVA). Significant differences between treatments (indicated by different letters) were confirmed by significant difference (LSD) testing at the 5% and 1% levels of significance. All statistical analyses were performed with SPSS 13.0 software (SPSS 13.0, Inc., USA).

Conclusions

The results of this study revealed that the levels bioactive components in roots of *S. miltiorrhiza* vary significantly according to the germplasm and with root characteristics such as the root color, diameter, and cortex/xylem ratio. DS007 from *Henan Yuzhou* contained large amounts of both hydrophilic (HC) and lipophilic (LC) components (i.e., DSS, PTAL, SAB, CTS, and TSIIA). The levels of DSS, SAB, CTS, and TSIIA were higher in the cortex (including the phloem and cork layers) than in the xylem. CTS and TSIIA were more abundant in the roots with smaller diameters. The CTS, TSIIA, and SAB levels were lowest in the lutou (basal or residual part of stem), while the DSS and PTAL levels were highest in this portion. The levels of both HC and LC were higher in more deeply colored (brownish-red) root powders. However, further exploration of the insights of the present work by examination of the biological and biochemical relationships between the BC contents and root structures, colors, and other root characteristics would be most interesting.

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